Stabilization of Linseed Oil for use in Aquaculture Feeds

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon, Saskatchewan

By

Stephanie A. Nilson

Fall, 2008

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may me granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any materials in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 5A8

ABSTRACT

An experiment was conducted to determine the effect of addition of antioxidants or encapsulation of linseed oil on the oxidative stability of linseed oil and the effect on growth and fatty acid composition of rainbow trout fed these products. Four diets differing only in their lipid sources were prepared by cold extrusion: 1) fish oil (FO), 2) linseed oil (LO), 3) linseed oil (980 g/kg) stabilized with vitamin E (7.5 g/kg) and butylated hydroxytoluene (BHT) (12.5 g/kg) (stabilized linseed oil; SLO) and 4) linseed oil (350 g/kg) containing vitamin E (7.5 g/kg), BHT (12.5 g/kg) and encapsulated in a coating material primarily consisting of hydrogenated palm oil (630 g/kg) (encapsulated linseed oil; ELO). Diets were fed twice daily to rainbow trout to apparent satiation (n=22 / replicate; 7 replicates per treatment) during a 168 day growth trial. Following the growth trial, the fish were humanely euthanized by a sharp blow to the cranium and analyzed for fatty acid composition, thiobarbituraric reactive substances (TBARS), fillet colour and sensory attributes (trained and consumer panels). There were no significant differences between treatments on any of the growth parameters investigated or TBARS levels of fish fillets. Omega-3 polyunsaturated fatty acids of trout fed LO were significantly higher than those fed FO (35.5% of total fatty acids vs. 27.6%) and ELO (28.9%) ($P < 0.05$). EPA and DHA levels were not significantly different between treatments. Diet samples were stored for 168 days at room temperature in sealed plastic containers. Following storage, the oxidative stability index (OSI) of the FO and LO diets were reduced to 0.00 hours while that of the SLO diet 9.20 hours and the ELO diet was 11.40 hours. Trained panelists determined fish fed FO had a significantly higher aroma

intensity and significantly lower aroma desirability and overall acceptability than those fed SLO. The rancid aroma and flavour of the FO-fed fish was significantly higher than fish fed the other treatments ($P < 0.05$). Consumer panelists found no significant differences between the sensory attributes of fish fed the four experimental diets and exhibited no preference between treatments ($P > 0.05$). Fillets from fish fed FO had significantly higher values than the other three treatments for redness (3.59 vs values between 1.86 and 2.07) and yellowness (25.35 vs values between 20.51 and 21.22) ($P < 0.05$). Addition of antioxidants to linseed oil improves its oxidative stability during storage and processing and results in fish fillets with fatty acid composition and consumer acceptance equal or superior to fish fed fish oil.

ACKNOWLEDGEMENTS

Thank you to my supervisor, Dr. Murray Drew and the members of my graduate committee: Dr. Bernard Laarveld, Dr. Fiona Buchanan, Dr. John McKinnon and Dr. Phyllis Shand for their guidance, assistance and patience. Thank you to my husband Michael, my parents Grant and Nadine Collins, my sister Adrienne Collins Bretell, my brother Preston Collins, my aunts Colleen Turtle and Cathy Almaas, my uncle Len Turtle and Haley, Willow, Ricochet, Spider and Zoe for their love and support.

Special thanks to Seth Briggs, Matthew Scobie and Nevada Young for their help with the growth experiment, Dr. Xiangfeng Meng and Dr. Darcy Driedger in Brooks for extruding the diets, Logan Williams, Grant Zalinko Renee Beliveau and Dr. Andrzej Olkowski for teaching me much of the fatty acid analysis methodology, Megan Halter and Nevada Young for helping with the fatty acid and TBARS analysis. Thanks to Dr. Shand and Heather Silcox for teaching me how to conduct a taste panel, Jeffrey Lindsay and Stephen Bohrson for aiding in carrying out the process and Dr. Drew, Dr. Shand and Dr. Zeb Pietrasik for helping with the statistical analysis. Thank you to Dr. Drew, Neil Wiebe, Heather Silcox, Grant Collins, Adrienne Collins Bretell, Nevada Young, Tanya Schafer, Tanya Warren, Dr. Benjamin Willing, Ryan Ridgeway, Stephen Bohrson, Dr. Jaipal Dahiya, Dr. Darryl Wilkie and Pam Kish for processing 500 fish in the period of two days. A special thank you also to everyone who participated in the taste panels.

Thank you to the Flax Council of Canada and the Natural Sciences and Engineering Research Council of Canada for their generous funding and to JEFO Nutrition Inc for providing the encapsulated linseed oil.

DEDICATION

This thesis is dedicated to the memory of my grandmothers, Mary Collins and Selena Turtle, both of whom passed away during my studies as a Master's student and both from whom I learned many life lessons, heard many stories and shared many hugs. This thesis is also dedicated to the memory of my grandfathers, Bill Collins and George Turtle, the former who I only knew for a short while and the latter, I never met. Stories of these people and their love of nature as well as their courage and strength in the face of adversity has helped lead me along the path to my interest in pursing a career in science and reminded me to be thankful for the things I have in my life, the most important being my family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

a*	Redness
AA	Arachidonic Acid
ALA	Alpha-Linolenic Acid
b*	Yellowness
BHT	Butylated Hydroxytoluene
COX-1	Cyclo-Oxygenase-1 Enzyme
COX-2	Cyclo-Oxygenase-2 Enzyme
DDT	Dichlorodiphenyltrichloroethane
DGLA	Dihomo-Gamma-Linolenic Acid
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
ELO	Encapsulated Linseed Oil
EPA	Eicosapentaenoic Acid
ETA	Eicosatetraenoic Acid
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration of the United States
FO	Fish Oil
GC	Gas Chromatography
GLA	Gamma-Linolenic Acid
HSI	Hepatosomatic Index
HUFA	Highly Unsaturated Fatty Acid

I_2	Iodine
L*	Lightness
LA	Linoleic Acid
LO	Linseed oil
LTB4	Leukotriene B4
LTB5	Leukotriene B5
MDA	Malonaldehyde
n-3	Omega-3
n-6	Omega-6
n-9	Omega-9
O_2^-	Superoxide
OSI	Oxidative Stability Index
pg	Picogram
PCB	Polychlorinated Biphenyl
PER	Protein Efficiency Ratio
PGE1	Series 1 Prostaglandins
PGE2	Series 2 Prostaglandins
PGE3	Series 3 Prostaglandins
POP	Persistent Organic Pollutant
PUFA	Polyunsaturated Fatty Acid
SDA	Stearidonic Acid
SEM	Standard Error of the Mean
SGR	Specific Growth Rate

SLO	Stabilized Linseed oil
TBARS	Thiobarbituric Acid Reactive Substances
TBHQ	*Tert*-Butylhydroquinone
TEQ	Toxic Equivalent
THA	Tetracosahexaenoic Acid
TPA	Docosatetraenoic Acid
VSI	Viscerosomatic Index

1. INTRODUCTION

Aquaculture is currently growing at an average annual rate of 6.5% and this rate is expected to continue until 2025 (Shepherd *et al*., 2005). This explosive growth in aquaculture has resulted in a concomitant growth of aquaculture feed production. Historically, aquaculture feeds have been based on two primary feed ingredients: fishmeal and fish oil, both of which have limited supply. The world's demand for fish oil already exceeds supplies (Shepherd *et al*., 2005) and by 2020, the same will be true for fishmeal (New and Wijkstrom, 2002). The imminent scarcity of adequate supplies of fish oil amplifies the necessity of finding a replacement for fishmeal and oil. Until this dilemma is solved, the sustainability of aquaculture is in jeopardy.

Fish oil has a unique fatty acid composition. It has a high content of long chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). These fatty acids are important for the proper growth and intermediary metabolism of aquaculture species (NRC, 1993). It is difficult to simply replace fish oil with another vegetable-based oil, as they lack EPA and DHA (NRC, 1993). Some fish have the capability to convert the fatty acid alpha-linolenic acid (18:3n-3; ALA) to EPA and DHA, although in previous research, it has been shown that replacing fish oil with vegetable oil at high levels results in fish containing lower tissue EPA and DHA concentrations (Caballero *et al*., 2002; Bell *et al*., 2003). Most vegetable-based oils are relatively low in ALA, with the exclusion of canola and linseed oils, which are both very high in ALA as a percent of total fatty acids and have an increased n-3:n-6 fatty acid ratio (NRC, 1993).

Fish tissue EPA and DHA concentrations are lower when fish oil in the diet is replaced with vegetable oil (Bell *et al*., 2001; Caballero *et al*., 2002; Rinchard *et al*., 2006). However, in turbot, it had been found that increasing the levels of ALA in the diet can improve the fatty acid bioconversion of ALA to EPA and DHA action can be taken to increase the conversion of ALA to these fatty acids (Leger *et al*., 1979). This is despite the fact that turbot generally have poor capabilities of synthesizing EPA and DHA (Owen *et al*., 1975). Increased EPA levels in the diet can negatively influence delta-5-desaturase activity (Mozaffarian *et al*., 2005), which is required to convert ALA to EPA (Buzzi *et al.*, 1997). Increased desaturase activity has been noted in salmonids fed diets where vegetable oil has replaced fish oil at high levels, despite decreased tissue EPA and DHA levels (Tocher *et al*., 2001; Tocher *et al*., 2004). Ascorbic acid, magnesium and zinc, vitamin and mineral cofactors have been shown to influence delta-6-destaturase activity (Horrobin, 1981; Gardiner and Duncan, 1988). These findings suggest that optimal conversion of ALA to EPA and DHA may best be encouraged via high dietary levels of ALA, low dietary levels of EPA and proper vitamin and mineral nutrition.

Maintaining high levels of EPA and DHA in fish is important as health-conscious consumers desire high levels of these PUFAs in their diets for their health benefits (Schmidt *et al*., 2005). Current Western diet n-3:n-6 fatty acid ratios have deviated to levels of 1:10 or higher, whereas hunter-gatherer diets are thought to have consumed diets at an n-3:n-6 fatty acid ratio of 1:2-3 (Cordain *et al*., 2005). An increased n-3:n-6 fatty acid ratio has been found to be associated with reduced risk of coronary heart disease (Harris *et al*., 2007), as well as reduced risk of a number of other diseases and ailments.

Although it may prove to be an important n-3 fatty acid to aquaculture nutrition in the future, ALA also poses its challenges. ALA is prone to oxidation and storage and processing can increase its rate of oxidation (Boran *et al*., 2006). Oxidation may decrease the nutritional value of ALA by decreasing it availability for bioconversion to EPA and DHA. Consumer acceptance of fillets of fish fed such oils may also be affected.

Antioxidants, such as such as vitamin E and butylated hydroxytoluene (BHT), may be added to oils to prevent their oxidation. Vitamin E reduces lipid peroxidation (Chaiyapechara *et al*., 2003; Lukaszewicz *et al*., 2004), serves antioxidant properties at levels above those required nutritionally and can improve the meat quality of fish (Waagbø *et al*., 1993). BHT is a synthetic analogue of vitamin E and fat-soluble (Wanasundara and Shahidi, 1994) it is used in a number of products, including animal feeds, cosmetics and pharmaceuticals (Shahidi and Wanasundara, 1992). Oxidation can also be prevented via encapsulation. Palm oil, acacia gum and hydrolyzed starches are some commonly utilized materials for encapsulation. Droplets of oil are coated, and are thus protected from environmental oxygen (Tawfik and Huyghebaert, 1999; Turchiuli *et al*., 2005).

The replacement of fish oil with vegetable oil while maintaining the composition of aquaculture fish products is the central problem in aquaculture nutrition today. The following experiment was performed to determine the effect of adding antioxidants to or encapsulation of linseed oil on the oxidative stability of pelleted diets, the growth of rainbow trout and the chemical and sensory properties of resulting fish fillets.

2. LITERATURE REVIEW

2.1 Biochemistry of lipids and fatty acids

A fatty acid is a carboxylic acid with an unbranched (usually), aliphatic chain. Fatty acids may be differentiated by: 1) their chain length, 2) number of double bonds and 3) the position of the double bonds in the aliphatic chain. When a fatty acid isomer is written numerically, the first number indicates the number of carbon atoms the molecule has and the second number states the number of double bonds in the molecule. Some numerical designations may be followed by "n-3" or "n-6." If the fatty acid molecules are n-3 (omega-3) or n-6 (omega-6) isomers, their first double bonds are three and six carbons from the methyl end of the carbon chain, respectively (Lee *et al*., 2007). Thus, a 18:3n-3 indicates a fatty acid with 18 carbon atoms and 3 double bonds with the first double bond after the 3rd carbon atom in the chain. Other possible isomers also exist. There may also be a "c" or a "t" to specify if a molecule is in cis or trans configuration. However, trans fatty acids are usually a byproduct of hydrogenation (Tavella *et al*., 2000) and do not generally occur in vegetable or fish oils.

Fatty acids are commonly classified as saturated or unsaturated. The term saturated is used to describe a fatty acid containing no double bonds, while the term unsaturated is used for any containing one or more double bonds. Unsaturated fatty acids may be monounsaturated or polyunsaturated. The more double bonds a fatty acid has, the more highly unsaturated it is. These double bonds are prone to oxidation (Kahl and Hildebrandt, 1986).

Polyunsaturated fatty acids (PUFAs) are long-chain unsaturated fatty acids

containing two or more double bonds and often serve important physiological functions. Very long-chain PUFAs with 3-6 double bonds such as EPA and DHA, containing chain lengths of 20 and 22 carbon-lengths, respectively, are referred to in some papers as highly unsaturated fatty acids (HUFAs) (Tocher *et al*., 2004; Zheng *et al*., 2004; Rinchard *et al*., 2007). There are three classifications of PUFAs discussed in this paper: n-3, n-6 and n-9. N-3 PUFAs include fatty acids such as ALA, EPA and DHA. These fatty acids are high in fish, linseed and walnut oils (Rinchard *et al*., 2006; Griel *et al*., 2007). N-6 PUFAs include fatty acids such as linoleic and arachidonic acid. These fatty acids are high in oils such as corn, soybean and sunflower oils (McLennan and Dallimore, 1995; Yoneda *et al*., 2007). Oleic acid is an n-9 fatty acid that can be found in oils such as tallow and lard (Haard, 1992).

Humans and fish species (as well as all other vertebrates) require linoleic acid (18:2n-6; LA) and ALA in their diets as they lack the enzymes required to desaturate oleic acid (18:1n-9) to these fatty acids (Holman, 1986; Rinchard, *et al*., 2007). Once supplied with LA and ALA, humans and non-marine fish species have the capability to synthesize arachidonic acid (20:4n-6; AA), EPA and DHA as well as a number of other metabolites from these precursors. This fatty acid biosynthesis takes place via a number of fatty acyl desaturation, elongation and oxidation processes (Figure 2.1) and occurs within the hepatocytes of the liver and the intestinal enterocytes (Tocher *et al*., 2001; Burdge *et al*., 2002; Tocher *et al*., 2004; Zheng *et al*., 2004).

In the body, ALA is converted to stearidonic acid (18:4n-3; SDA) by the delta-6-desaturase enzyme, which removes two hydrogen atoms, creating another double bond. Elongation then occurs. SDA is converted to eicosatetraenoic acid (20:4n-3; ETA) by the

elongase enzyme by adding another two carbon atoms. Eicosatetraenoic acid is converted to EPA (20:5n-3) by the delta-5-desaturase enzyme, which removes another two

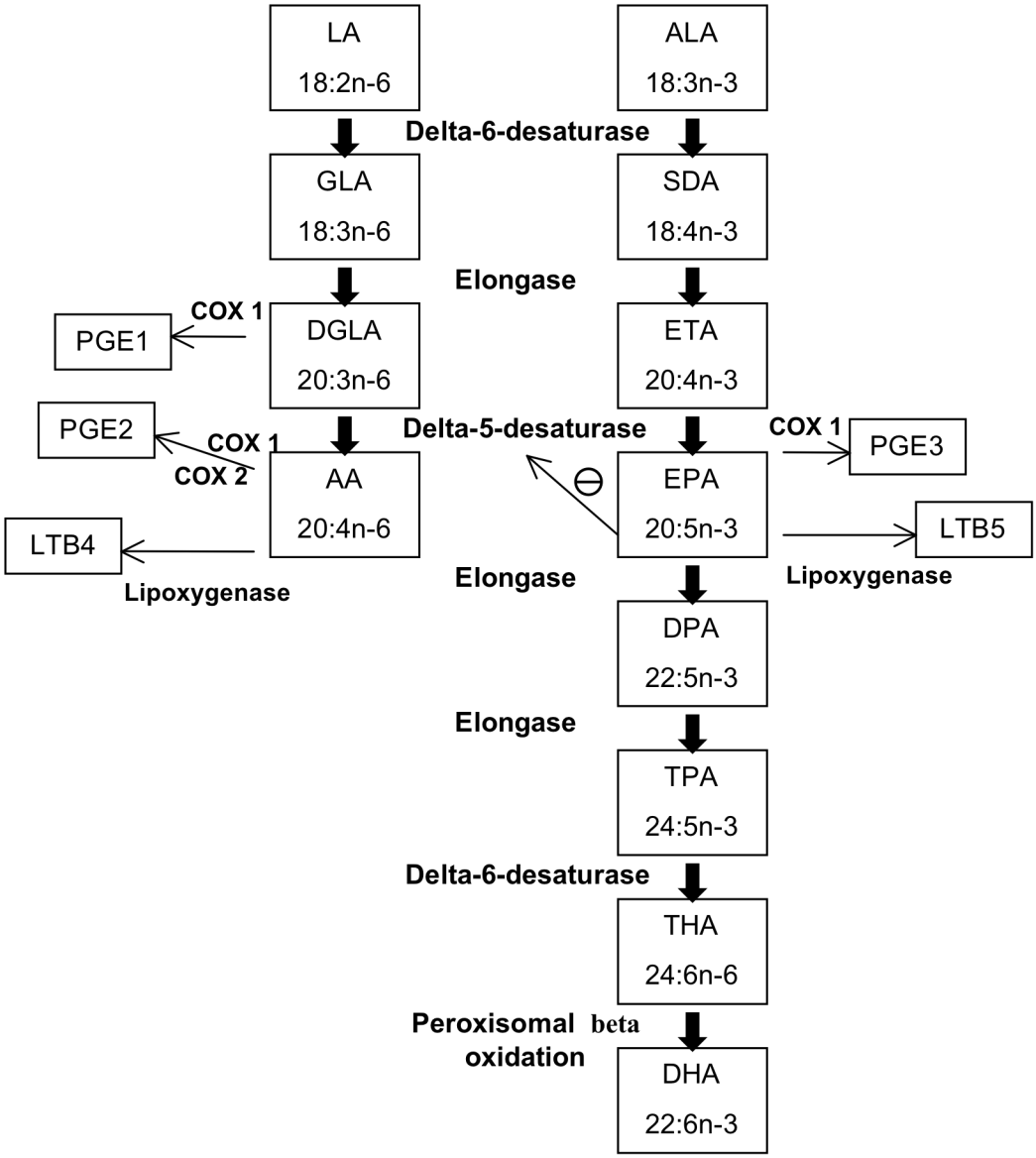

Figure 2.1 Metabolism of essential fatty acids. After Kragballe *et al*., 1987; Buzzi *et al.*, 1997; Yang *et al*., 2004; Mozaffarian *et al*., 2005.

hydrogen atoms, creating a fifth double bond. At the same time, a similar reaction occurs for LA, utilizing the same processes. Delta-6-desaturase converts LA to gamma-linolenic

acid (18:3n-6; GLA). The elongase enzyme converts gamma-linolenic acid to dihomo-gamma-linolenic acid (20:3n-6; DGLA). Delta-5-desaturase converts DGLA to arachadonic acid (20:4n-6; AA) (Nakamura and Nara, 2003; Mozaffarian *et al*., 2005).

DHA is formed from EPA. EPA goes through chain elongation in the liver microsomes to form docosapentaenoic acid (22:5n-3; DPA) then docosatetraenoic acid (24:5n-3; TPA). 24:5n-3 is desaturated by delta-6-desaturase to form tetracosahexaenoic acid (24:6n-3; THA). Peroxisomal beta-oxidation then causes chain shortening, resulting in DHA (22:6n-3) (Buzzi *et al.*, 1997).

The fatty acids EPA and AA may also be converted to a number of eicosanoids, comprised of prostaglandins, thromboxanes and leukotrienes, which regulate inflammation in the body (Williams and Peck, 1977; Garg *et al*., 1990; Mozaffarian *et al*., 2005). EPA converts to series 3, anti-inflammatory prostaglandins (PGE3) with the cyclo-oxygenase-2 (COX-2) enzyme (Yang *et al*., 2004). The lipoxygenase enzyme may also convert EPA into the (less-inflammatory) leukotriene B5 (LTB5) (Kragballe *et al*., 1987).

The pathway for n-6 fatty acids continues on to the series 1, anti-inflammatory prostaglandins (PGE1) and the series 2, pro-inflammatory prostaglandins (PGE2). PGE1 is derived from DGLA and AA is the precursor for PGE2. The enzyme cyclo-oxygenase-1 (COX-1) increases the production of PGE2 and the COX-2 equally produces PGE1 and PGE2 (Levin *et a*l., 2002). The (more-inflammatory) leukotriene B4 (LTB4) is derived from AA via the lipoxygenase enzyme (Kragballe *et al*., 1987).

Under normal conditions, the desaturase and elongase enzymes metabolize n-3 fatty acids before n-6 fatty acids, and n-6 before n-9 fatty acids. High levels of n-9 fatty

acids metabolites are indicative of an essential fatty acid deficiency (Das, 2006). When n-3 fatty acids are deficient or when n-6 fatty acids are consumed beyond requirements, the AA:EPA ratio of the diet increases. As the AA:EPA ratio increases, so do PGE2 levels, thromboxanes, leukotrienes (Simopoulos, 2002) and proinflammatory cytokines (Kiecolt-Glaser *et al*., 2007). Further exacerbating this is that AA is a more efficient PGE2 precursor than EPA is for PGE3 (Raisz *et al.*, 1989).

An increase of n-3 fatty acids in the diet prevents an increase of AA levels by increasing EPA levels, which block delta-5-desaturase activity (Mozaffarian *et al*., 2005), limiting PGE2 formation (Barham *et al*., 2000) and thromboxane production (Garg *et al*., 1990). When less DGLA is converted to AA, more DGLA is available for conversion to PGE1 although the conversion from DGLA to PGE1 is less efficient than the conversion of AA to PGE2 (Levin *et al*., 2002). In some cases, the AA:EPA ratio may be more relevant than the n-6:n-3 ratio as these are the two fatty acids competing for conversion to bioactive molecules (Harris *et al*., 2007).

The rate-limiting step of HUFA biosynthesis is due to a deficiency of delta-6-desaturase (Brenner, 1981). It is therefore important to ensure that delta-6-desaturase activity is maximized. For example, cofactors shown to have an effect on delta-6-desaturase by Horrobin (1981) are magnesium and zinc. The presence of ascorbic acid in the diet has been known to increase PGE1 production. It has also been shown to increase delta-6-desaturase activity (Gardiner and Duncan, 1988). In humans, delta-6-desaturase is also known to decrease with age and increase in accordance with a moderate decrease in food intake (Horrobin, 1981). Thus, vitamin and mineral nutrition may significantly affect the production of highly unsaturated fatty acids.

2.2 Lipid requirements of salmonid fish

As with all animals, fish have specific requirements for protein, energy, vitamins, minerals and lipids. What differentiates fish from other animals is that there are extreme species differences in requirements for these nutrients. This is largely due to the great number of fish species in existence as well as the huge variety in environments in which different fish species reside. They have adapted to live in different water salinity levels and water temperatures. As the primary lipid source used in aquaculture is fish oil, there is increased interest in determining exact lipid requirements of fish in order to find a suitable fish oil replacement.

Although lipids provide an energy source (Geurden *et al*., 2006), this is not the only reason they are required in aquaculture diets. Lipids are also a source of fat-soluble vitamins and essential fatty acids. Essential fatty acids are used to produce hormone-like substances, such as prostaglandins and leukotrienes that have a number of regulatory functions including immune and inflammation response. The fatty acids in the diets of fish can affect the fatty acid profile of their tissues (Fonseca-Madrigal *et al*., 2005), which is important in human nutrition.

Fatty acid conversion efficiency varies between fish species, with some converting ALA to stearidonic acid more efficiently than others (Zheng *et al*., 2004). This could be due to the environments in which they live as well as due to evolutionary adaptations of each particular species of fish. Different species of fish have varying abilities to synthesize longer chain fatty acids such as eicosapentaenoic and docosahexaenoic acid using the essential fatty acids supplied in the diet (Kanazawa *et al*., 1979; Tocher *et al*., 2001; Zheng *et al*., 2004), as described in Chapter 2.1.

If the EPA and DHA requirements of fish are greater than their ability to synthesize them, the balance must also be provided in the diet (Owen *et al*., 1975). The composition of a diet is not the only factor influencing fatty acid bioconversion. Fatty acid desaturase activity is also determined by water temperature (Tocher *et al.*, 2004) and nutritional factors. In salmonid fish, feeding high vegetable oil diets as opposed to high fish oil diets can increase desaturase activity (Tocher *et al*., 2001; Tocher *et al*., 2004).

Rainbow trout and other salmonids are capable of biosynthesizing long-chain fatty acids, providing there are adequate levels of alpha-linolenic acid in the diet. The rate of biosynthesis varies between salmonids. Tocher *et al*. (2001) investigated the efficiency of biosynthesis of fatty acids in three salmonid species (brown trout, Arctic charr and Atlantic salmon) and found there were significant differences between the three species. Other fish, such as turbot have reduced capabilities of synthesizing EPA and DHA (Owen *et al*., 1975), although increasing the level of ALA in the diet has been shown to improve its conversion to EPA and DHA (Leger *et al*., 1979). Essential fatty acid deficiency in rainbow trout results in reduced growth rate, heart myopathy, fin erosion, enlarged livers and loss of consciousness when stressed and occasionally death (Castell *et al*. 1972).

2.3 Fish Oil

The aquaculture feed industry is highly dependent on fish oil generated from the global fisheries. Total production of fish oil is approximately 1.4 million tonnes per year (Shepherd *et al*., 2005) and this level of production is expected to remain constant or decrease slightly in the future. Fish oil has been the primary lipid source in aquafeeds over the last 40 years (New and Wijkstron, 2002).

Fish oil is high in fatty acids that are not common in many other plant and animal-based oils. Fatty acids in fish oil that are commonly considered to be highly desirable for both use in aquaculture and in human nutrition include polyunsaturated fatty acids such as EPA and DHA. As can be seen in Table 2.1, fish oil generally contains high levels of 20:5n-3, 22:6n-3 and relatively low levels of 18:2n-6 and 18:3n-3. However, different fish oils can vary in specific levels of these essential fatty acids.

Table 2.1 Fatty acid composition of fish oil

	Percent fatty acid in oil*							
Source	**18:2n-6**	**18:3n-3**	**20:5n-3**	**22:6n-3**	**n-6**	**n-3**	**n-3:n-6**	**n-6:n-3**
Anchovy	1.2	0.8	17.0	8.8	1.3	31.3	24.0	0.0
Atlantic herring	1.1	0.6	8.4	4.9	1.4	17.8	12.7	0.1
Capelin	1.7	0.4	4.6	3.0	1.8	12.2	6.8	0.1
Menhaden	1.3	0.3	11.0	9.1	1.5	25.1	16.7	0.1
Salmon	1.2	0.6	12.0	13.8	2.1	31.4	15.0	0.1

*Source: NRC, 1993

2.4 Fish in Human Diets.

2.4.1. Fish as a source of fatty acids

EPA and DHA levels are higher in fish and other seafood products as compared with other meat and vegetable sources (Howe *et al*., 2006). There is debate as to whether farmed or wild fish provide greater health benefits. In some instances, farmed fish generally contain lower levels of omega-3 fatty acids than wild fish (Alasalvar *et al*., 2002), although not always. In halibut, it was found by Olsson *et al*. (2003) that wild fish had a higher percent composition of EPA and DHA, although the level of these fatty acids per 100 g of muscle was higher in farmed fish. There are improvements being made

in the use of fatty acids in aquaculture. Studies have shown it is possible to produce fish by feeding low n-3 feeds early in growth and a high n-3 finishing diet and result in the same high n-3 product that would be obtained from feeding a high n-3 diet through the entire growth cycle (Bell *et al*., 2003; Bell *et al*., 2004). This results in a lower cost of feeding and a reduced impact on fish stocks.

2.4.2. Consumption of fish in human diets

The public is aware of the importance of consuming fish for health benefits, particularly for its omega-3 content. It is estimated hunter-gatherer diets consisted of an n-6 to n-3 fatty acid ratio of 2-3:1. Common Western diets have deviated from this original diet and are composed primarily of what were previously novel foods. This can lead to n-6 to n-3 fatty acid ratios as high as 10:1 (Cordain *et al*., 2005). This fatty acid ratio could be considered an improvement, as in 1985, people living in the United States consumed n-6 and n-3 fatty acids in an average ratio of 12.4:1, and this dropped to 10.6:1 in 1994 (Kris-Etherton *et al*., 2000). The recommendations for the daily intake of EPA and DHA were recently increased from 0.1-0.2 g/d to 0.65 g/d in the United States (Kris-Etherton *et al*., 2000). The authors stated that to meet the requirement of 0.65 g/d of EPA and DHA would require a four-fold increase in fish consumption in the US. Given that fish is the primary source of EPA and DHA in human diets, maintaining the fatty acid composition of aquaculture fish is a critical issue in human health.

2.4.3. Health benefits of eating fish

There are a number of health benefits that are thought to be associated with consuming

fish, or more appropriately, consuming EPA and DHA, thus decreasing the n-6 to n-3 fatty acid ratio. Diets consisting of reduced linoleic acid and higher levels of EPA and DHA have been shown to result in reduced coronary heart disease (Harris *et al*., 2007). As their consumption has been associated with a decreased risk of coronary heart disease, n-3 fatty acids are considered cardioprotective (Mozaffarian *et al*., 2005).

The PUFAs found in fish do not provide health benefits solely to the heart. There are a number of physical conditions thought to be prevented or improved by the consumption of EPA and DHA. Adequate intake of n-3 fatty acids may also result in improved bone health (Griel *et al*., 2007), a reduced risk of premenopausal breast cancer (Goodstine *et al*., 2003) and reduced risk of inflammatory diseases and depression (Kiecolt-Glaser *et al*., 2007) as well as many others.

2.4.4. Contaminants in farmed fish

Aquafeeds contain high levels of fishmeal and fish oil. This meal and oil is obtained from marine food chain sources. Often pelagic fishes, such as herring, capelin, sandeel and menhaden are used in fishmeal (Hevrøy *et al*., 2004; Li *et al*., 2004), as opposed to the invertebrates that would make up the natural diet of some of these farmed fish species (ex. salmonids). For example, Aydin *et al*. (2005) observed the natural diet of pink salmon to consist primarily of zooplankton and small squid. These species are much lower in the food web than those commonly fed in aquaculture. This practice causes fish raised in aquaculture to be fed at a higher trophic level than they would naturally eat at in the wild.

Heavy metals such as mercury, lead, cadmium, iron, copper, zinc, cadmium,

manganese, silver, aluminum, arsenic, chromium, nickel, selenium and zinc can be found in fish at various levels (Alam *et al*., 2002; Dehn *et al*., 2006; Ureña *et al*., 2007; Türkmen *et al*., 2008) and are known to bioaccumulate as they move up the food chain. The significance of this bioaccumulation varies between animals and is determined by the diet of the animal as well as by other biological factors of the animal (Dehn *et al*., 2006).

The geographical location fishes originate from can determine the types and levels of heavy metals in their tissues (Türkmen *et al*., 2008). Other determining factors include longevity, size and feeding habits of fishes. Predatory, long-lived fishes such as tuna and bottom-feeding fishes such as flounder generally contain higher heavy metal levels than shorter-lived (albeit predatory) fishes such as cod (Burger and Gochfeld, 2005). In separate studies conducted by Alam *et al*. (2002) on the European carp (an herbivore / invertivore) and Ureña *et al*. (2007) on the European eel (a carnivore), there were few differences in levels of heavy metals between wild fish and those raised via aquaculture and all at levels considered safe for human consumption.

Fish can contain toxic substances known as persistent organic pollutants (POPs). They bioaccumulate as they move up the food chain (IPCS, 1992). When consuming a standard European diet, the danger of consuming a dangerous level of POPs in a short period of time is low (Harrison *et al*., 1998). It is prolonged periods of consumption that are most likely to result in negative effects, as POPs persist in tissues for long periods of time and are found to have numerous toxic effects at high levels (Tsai *et al*., 2007).

Farmed fish tend to contain higher levels of POPs than wild-caught fish. Some of these persistent organic pollutants include: polychlorinated biphenyl (PCB), the well-known organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and its

derivatives, as well as many others (Easton *et al*., 2002; Antunes and Gil, 2004; Carubelli *et al*., 2007). Production of these pollutants has been halted or controlled since the 1970's due to evidence of environmental harm. Nevertheless, POPs persist and are still released into the environment via landfills, spills and illegal dumping (IPCS, 1992).

Many POPs are lipophilic (Startin *et al*., 1989), explaining their presence in fishmeal and fish oil. Higher POPs in farmed fish as opposed to wild-caught fish are likely due to high POP levels in their feed (Easton *et al*., 2002; Antunes and Gil, 2004; Carubelli *et al*., 2007; Maule *et al*., 2007). Farmed fish tend to have a higher body fat composition than wild fish, which may also be responsible for their higher tissue POP levels (Carubelli *et al*., 2007). POPs are also found in more commonly consumed meat sources such as beef, pork and poultry, although generally at lower levels. Schecter *et al*. (1998) compared the levels of POPs (specifically, dioxins, dibenzofurans and coplanar PCBs) in raw and cooked hamburger, bacon and catfish fillets. They found these POPs were present in hamburger, bacon and catfish fillets at mean concentrations of 155, 145 and 378 pg TEQ (toxin equivalents) /kg meat, respectively. Following broiling for approximately 30 minutes, the concentrations were reduced to mean values of 16, 8 and 55 pg TEQ /kg meat for hamburger, bacon and catfish fillets, respectively. Kim *et al*. (2004) compared three coplanar PCBs (PCB-77, PCB-126, and PCB-169) in pork, beef and chicken fat and found the sum of these three PCBs present at mean levels of 7.13 pg TEQ /kg for the pork, 9.71 pg TEQ /kg for the beef and 9.30 pg TEQ /kg for the chicken.

In pregnant and breastfeeding women, POPS can cross the placenta to the fetus and can be passed on to the infant via breast milk (Startin *et al*., 1989; Harrison *et al*., 1998). Exposure to POPs can determine the health of these infants for the rest of their

lives. Adults who consume high levels of POPs are not without risk. The specific POP consumed will dictate the major negative effects incurred (Tsai *et al*., 2007).

Plant oil sources are being investigated to determine viable, lower POP fish oil replacements. Drew *et al*. (2007) determined replacing fish oil with canola and linseed oil reduced the presence of several POPs in rainbow trout fillets. This indicates replacement of fish oil with vegetable oils in aquafeeds can increase the safety and health benefits of fish raised in aquaculture.

2.5 Taste preference

Fish are an excellent source of n-3 fatty acids in human diets and provide measurable health benefits. However, consumption of food products is generally based on taste and other sensory preferences rather than nutritional content. The consumer may be deciding between fish and another protein source, such as poultry or beef. Many sensory factors determine whether or not a consumer will find a particular fish product acceptable for consumption. Some include (but are not limited to) flavour, aroma, colour, appearance, consistency, texture, hardness, compactness, juiciness and roughness (Rørå *et al*., 1998; Regost *et al*., 2003; de Francesco *et al*., 2004). Dietary fat is a primary factor in determining the flavour and aroma of fish products (Grigorakis, 2007). Thus replacement of fish oil with vegetable oil might significantly affect consumer acceptance of fish products.

2.6 Replacement of fish oil with vegetable oils

As previously mentioned, fish oil has been the staple lipid source in aquafeeds for the last

40 years (New and Wijkstrom, 2002). It meets the nutritional requirements of fish for essential fatty acids and energy and creates fish products high in desirable PUFAs such as EPA and DHA. However, the worldwide shortage of fish oil has resulted in the necessity of using other lipid sources in aquafeeds.

Plant oil sources that are inexpensive, easy to procure and produce and meet the dietary needs of the aquaculture species to which they are being fed are the primary objective in the search for a fish oil replacement. Some of the plant-based oils currently being investigated for the replacement of fish oil in aquafeeds include linseed (flaxseed) oil, canola (rapeseed) oil, palm oil and sunflower oil. Most oils contain relatively low levels of n-3 fatty acids (Table 2.2) in comparison with fish oil (although there are some exceptions) and all are completely devoid of EPA and DHA (Bell *et al*., 2001; Bell *et al*., 2004; Fonseca-Madrigal *et al*., 2005; Geurden *et al*., 2006; Rinchard *et al*., 2006). The capability of some fish to biosynthesize EPA and DHA from ALA has many researchers investigating the use of or sustainable, plant-based oils to replace fish oil in aquaculture diets.

Table 2.2 Fatty acid composition of plant-based oil

	Percent fatty acid in oil*							
Source	**18:2n-6**	**18:3n-3**	**20:5n-3**	**22:6n-3**	**n-6**	**n-3**	**n-3:n-6**	**n-6:n-3**
Canola	20.2	12.0	-	-	20.2	12.0	5.9	1.7
Corn	58.0	0.7	-	-	58.0	0.7	0.0	82.9
Linseed	12.7	53.3	-	-	12.7	53.3	4.2	0.2
Palm	9.1	0.2	-	-	9.1	0.2	0.0	45.5
Soybean	51.0	6.8	-	-	51.0	6.8	0.1	7.5
Sunflower	65.7	-	-	-	65.7	0.0	0.0	0.0

*Source: NRC, 1993

As can be seen in Table 2.2, canola and linseed oil both contain high levels of 18:3n-3 and lower levels of 18:2n-6 than most of the plant-based oils as a percent of total fatty acid content. Linseed oil contains the highest levels of 18:3n-3. Linseed and canola oils are similar to fish oil in that they contain a more similar n-3:n-6 fatty acid ratio than any other vegetable oils. For example, the highest n-3:n-6 ratio for the fish oils listed in Table 2.1 is anchovy oil at a level of 24.0. The lowest is for capelin oil, which has a n-3:n-6 ratio of 6.8. The n-3:n-6 ratios of linseed and canola oil as listed in Table 2.2 are 4.2 and 0.59, respectively. These ratios are quite low as compared with the anchovy oil but much closer in value than any of the other vegetable oils listed in Table 2.2.

There has been extensive research in feeding vegetable oils to aquaculture species to determine their effect on fish fatty acid composition. Most research has resulted in the conclusion that fish oil may be partially replaced by vegetable oils such as linseed oil, rapeseed oil and palm oil without affecting the growth or health of the fish. However, when fish oil in the diet is replaced at high levels by vegetable oil, fish tend to have reduced levels of 20:5n-3 and 22:6n-3 and a lower n-3:n-6 ratio than those fed a high fish oil diet (Bell *et al*., 2001; Bell *et al*., 2002; Bell *et al*., 2004; Rinchard *et al*., 2006).

Linseed oil contains higher linoleic acid levels than fish oil, and thus it is more readily oxidized if stored or handled improperly (Lukaszewicz *et al*., 2004). Its tendency to oxidize is the reason for its application in products such as paints and linoleum. This propensity towards oxidation raises concern for its use in aquafeeds, as it may affect its nutritional properties and impart a painty flavour or aroma to the fish. It is therefore essential to prevent the oxidation of linseed oil during feed production and storage.

2.7 Lipid peroxidation

Lipid peroxidation is a degenerative process lipids undergo due to oxidation. Polyunsaturated fatty acids are especially susceptible to lipid peroxidation (Fernández *et al*., 1997). There are three major steps to lipid peroxidation: initiation, propagation and termination.

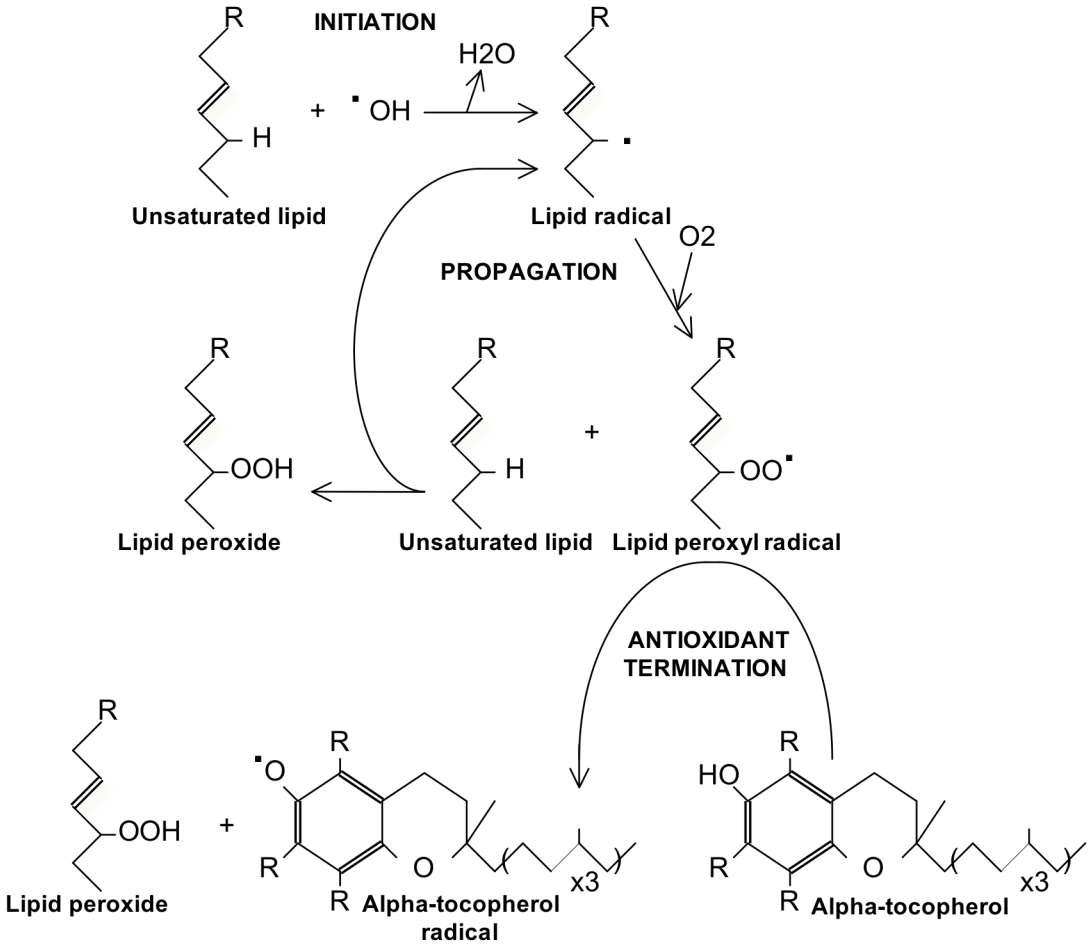

Figure 2.2 Lipid peroxidation and peroxide termination by antioxidant activity. After Kelly *et al*., 1998 and Young and McEneny, 2001.

In initiation, a fatty acid radical is produced (Shahidi and Wanasundara, 1992). A hydrogen atom on the fatty acid molecule is removed via hydrogen abstraction, resulting in water and a fatty acid radical (Khal and Hildebrandt, 1986). Light and heat act as catalysts in this process (Fernández *et al*., 1986; Kahl and Hildebrandt, 1986). In propagation, the fatty acid radical is oxidized, forming a lipid peroxy radical, which acts on another PUFA. This cycle continues and increases more and more rapidly as the reaction progresses until the lipid is completely oxidized or the reaction is terminated (Kahl and Hildebrandt, 1986). Termination occurs when two radicals react, producing a non-radical species. For this, radicals must be present in high enough concentration and must meet in the correct orientation. An antioxidant (such as vitamin E, or alpha-tocopherol) may also interfere, causing termination (Shahidi and Wanasundara, 1992).

Peroxidation of oils in aquafeeds may lead to byproducts that negatively affect the palatability and health benefits of these diets (Fontagné *et al*., 2006). Oils in aquafeeds are known to affect the carcass quality of the fish to which they are fed (Bell *et al*., 2001). Oxidized fatty acids in the diet can affect carcass quality and fatty acid composition. In fish, they can have a negative impact on the sensory attributes and reduce the health benefits of eating fish (Kanner and Rosenthal, 1992).

2.8 Accelerators of lipid peroxidation

Lipid peroxidation reduces oil quality. Vegetable-based oils may oxidize in storage, making them unappealing to the aquaculture industry, as a longer shelf life is desirable for aquafeeds. Fish oil experiences similar problems with oxidizing fatty acids (Boran *et al*., 2006). There are specific factors that can accelerate the oxidation process,

significantly reducing the shelf life of many oils. Avoiding them can increase the keeping quality of oils.

Exposure to oxygen, heat and light can accelerate the oxidative process of oils (Shahidi and Wanasundara, 1992; Ulu, 2004). Storing oils at warmer temperatures and exposure to heat produced during extrusion can lead to lipid oxidation. The amount of oxygen an oil is exposed to can affect its degree of oxidation. Oils exposed to environmental oxygen are more susceptible to oxidation than those stored in a sealed container (Shahidi and Wanasundara, 1992).

2.9 Methods used to detect lipid peroxidation

Lipid oxidation involves a complex set of reactions, which may differ depending on the conditions under which a lipid is stored. This is why no one method has been developed for the detection of lipid oxidation (Kaya *et al*., 1993). There are a number of testing methods available, some more suitable for specific samples than others. It is often wise to perform several different methods on the same sample, as this will increase the validity of the procedure as a whole. With some samples, there may be interferences involved with a particular assay that would provide inaccurate results. Using several different assays would provide a certain amount of backing to the results obtained by each individual assay. Of the various methods used to test for lipid oxidation, there are two main categories: 1) predictive tests or 2) oxidation indicator tests.

2.9.1. Predictive tests

Predictive tests are assays that are used for samples that could potentially become

oxidized. Their purpose is to determine the amount of oxidation that could potentially occur in a sample. These predictive values can be used to make inferences on how the oils in a sample would react under given conditions. Two commonly used predictive tests are the oxidative stability index and the iodine value assay.

2.9.1.1. Oxidative stability index

The Oxidative Stability Index (OSI) is a test used to predict the amount of oxidation that could possibly occur in a sample. It is a measurement depicted in units of time (hours) required for the sample to reach a maximum point of oxidation. The longer it takes for the sample to be completely oxidized, the more stable the sample. At a constant temperature (110 ^{o}C), air is passed through an oil sample. This air causes the triglycerides in the sample to degrade into volatile acids. These acids will travel on the stream of air to water within a conductivity cell. It is in this water that the acids will be solubilized. These acids will ionize increasing the conductivity of the cell. A computer will monitor this conductivity and at a point referred to as the induction point, the conductivity of the cell will drastically increase. This induction point is correlated with the complete oxidation of the oil sample. After this increase in conductivity is complete, the time period between the start of analysis and the induction point will be recorded, resulting in the OSI time (Isbell *et al*., 1999).

The OSI is a widely accepted method used to measure the stability of a fat. There are currently two different instruments that are available commercially to perform these tests: the Rancimat (Brinkmann Instrument, Westbury, NY) and the Oxidative Stability Instrument (Omnion, Inc., Rockland, MA) (Tan *et al*., 2002). This method is considered

accurate enough that it has been accepted by the American Oil Chemists Society (AOCS, 1998). The use of these machines and procedures laid out by such a society will help ensure reproducibility of this experiment over time and through separate laboratories.

Experiments conducted by Tan *et al*. (2002) have shown that the use of a differential scanning calorimeter (Perkin-Elmer DSC-7, Norwalk, CT) is also effective in measuring OSI. This method is useful, as it does not require the use of chemicals whereas the other OSI measurement devices do. However, it does not provide an indication of the reproducibility of this method by more than one set of researchers.

2.9.1.2. Iodine value

The iodine value is based on reactions between iodine and fatty acids. The iodine number indicates the level at which a fat is unsaturated (Glushenkova and Markman, 1970). A higher iodine value indicates a higher level of unsaturated fats, thus more sites available for oxidation. Iodine values are measured in g of I_2 / 100 g of oil (Tan *et al*., 2002). Isbell *et al*. (1999) found that there was a relationship between the iodine value and the antioxidant activity of oil. This is also a method that has been accepted and standardized by the American Oil Chemists Society (Isbell *et al*., 1999).

2.9.2. Oxidation indicator tests

As predictive tests are used to determine the amount of oxidation that could potentially occur in a sample, oxidation indicator tests are used to determine the amount of oxidation that has already occurred (Kaya *et al*., 1993; Guillén and Cabo, 2002; Lukaszewicz *et al*., 2004). These can be used to determine the quality of oil in a sample during any given

point in time and make decisions based on these results. Three commonly used oxidation indicator tests are the thiobarbituric acid reactive substances assay, the peroxide value assay and the anisidine value assay.

2.9.2.1. Thiobarbituric acid reactive substances

The presence of thiobarbituric acid reactive substances (TBARS) in a sample of meat indicates that lipid peroxidation has taken place. The level of TBARS shows the amount of peroxidation that has already occurred (Lukaszewicz *et al*., 2004). The main thiobarbituric acid reactive substance that is measured is the compound malonaldehyde, which is a secondary product formed as a result of lipid peroxidation (Ulu, 2004). TBARS values tend to have a good correlation with sensory testing when being used to detect rancidity of foods (Fernández *et al*., 1997; Rhee and Myers, 2003; Campo *et al*., 2006), making it a good choice of an assay to pair with sensory testing.

This test can also detect aldehydes, alk-2-enals and alk-2,4-dienals produced during lipid oxidation, although malonaldehyde is the primary substance that is detected with this assay. Thiobarbituric acid reacts with these substances to produce a red colour that is measured using a spectrophotometer (at 532 nm) (Inoue *et al*., 1998). Although this assay can detect other substances, it was originally designed to detect malonaldehyde (Ulu, 2004).

This assay can have problems associated with it. If performed too soon, it will not detect all of the malonaldehyde, as some of it has yet to form or it may not detect all of the malonaldehyde if some is lost during the processing and / or storage process. Levels also begin to decline following a peak in lipid oxidation (Fernández *et al*., 1997). Another

problem is that some TBARS assays employ the use of heat, which may increase the level of lipid oxidation of the sample producing results that are higher than the actual values. If this assay is employed, it is best to use a method that is quick and involves a minimal use of heat. It is also beneficial to add an antioxidant to the sample prior to beginning testing to ensure that none of the oxidative products are due to the processing methods being used (Ulu, 2004).

2.9.2.2. Peroxide value

The peroxide value of a sample indicates the concentrations of peroxides and hydroperoxides that are produced during the early stages of lipid oxidation. The peroxide values are monitored for a sample and when it sharply increases, it indicates the end of the shelf life for that sample. Peroxide values are measured in terms of milli-equivalents per kg of sample. The main use of a peroxide value is to determine the quality of an oil sample. It is not able to indicate which secondary products of lipid oxidation are present and at what levels (Kaya *et al*., 1993). If these values are desired, a different assay would be more appropriate.

High values are indicative of a high level of lipid peroxidation. Low peroxide values can be indicative of low levels of lipid peroxidation or they may be depicting the peroxides that have not yet diminished following a peak of much higher concentrations. Guillén and Cabo (2002) noted that peroxide values of different oil samples increased to a maximum level of oxidation over a period of time, following which the peroxide values decreased. As well, for some oil samples, this value increased slowly until just before the peroxide value peaked. At this point, the peroxide value increased at a much higher rate.

Caution would have to be taken in order to ensure that the maximum peroxide value be measured.

2.9.2.3. Anisidine value

The anisidine value is determined by the level of reaction that occurs between anisidine and the non-volatile portion of a fatty acid that remains following the breakdown of hydroperoxides. A higher anisidine value indicates a greater level of lipid oxidation has occurred. The anisidine value is used to determine the amount of secondary oxidation products (such as alpha and beta-alkenals) that are in a sample due to lipid oxidation. However, the rate of hydroperoxide production does not always coincide with the production of secondary oxidation products (Guillén and Cabo, 2002).

To determine the anisidine value, a solution is made by combining 1g lipid with 100 ml isooctane / acetic acid / *p*-anisidine reagent. This solution is analyzed on a spectrophotometer at 350 nm. The anisidine value is calculated by multiplying the absorbance of the solution by 100 (Labrinea *et al*., 2001). This assay can be useful to use along the peroxide value assay (Labrinea *et al*., 2001) and the TBARS assay, as it can detect accurate levels of oxidation even if aldehydes are lost during processing. In this sense, it could be a good way to validate the results obtained from a peroxide value assay or a TBARS assay.

2.10 Prevention of lipid peroxidation

Lipids may be prevented from oxidizing by reducing their exposure to heat, light and oxygen (Shahidi and Wanasundara, 1992; Ulu, 2004). Vacuum packaging can reduce

exposure to oxygen. This may also be accomplished by storing oil in a well-sealed container with a layer of inert gas, such as nitrogen, passed over the oil. Refrigeration or freezing can also be employed (Shahidi and Wanasundara, 1992).

Often over long periods of storage or during stressful procedures to the oil, such as extrusion, further measures are required to keep oils from oxidizing. Antioxidants are universally used to prevent oils from going rancid. There are also newer protective methodologies being investigated. One such process is protection of the oil by encapsulating it within a highly saturated fatty acid, reducing its exposure to oxidation.

2.10.1. Antioxidants

Antioxidants may be added to lipids to prevent them from oxidizing. Phenolic antioxidants (chain-breaking antioxidants) bind with free radicals, initiating their termination step of lipid peroxidation. Other antioxidants (protective antioxidants) scavenge oxygen, preventing lipid peroxidation (Shahidi and Wanasundara, 1992). There are antioxidants naturally present in the body. Their function is to control the level of oxidative byproducts caused by cellular metabolism.

Superoxide dismutase is a chain-breaking antioxidant present in the cytoplasm that converts superoxide (O_2^-) to oxygen and hydrogen peroxide. Superoxide dismutase contains metal ions at the active site. Copper-zinc superoxide dismutase is found in the cytoplasm and nucleus and manganese superoxide dismutase can be found in the mitochondria. There is also an extracellular superoxide dismutase (Gu and Hecht, 1996; Imai and Nakagawa, 2003). Catalase acts as a protective antioxidant. It is an enzyme that is found in the peroxisomes (Imai and Nakagawa, 2003) and converts hydrogen peroxide

to oxygen and water (Vetrano *et al*., 2005). Glutathione peroxidase is an enzyme found in the peroxisomes that converts hydrogen peroxide to water and hydroperoxides to alcohols (Gu and Hecht, 1996; Li *et al*., 2000). There are four known types of glutathione peroxidase, all of which contain selenium at the active site (Imai and Nakagawa, 2003).

Some antioxidants are commonly added to food and feed products. Common examples include butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin, propyl gallate, vitamin E and vitamin C. There is concern some antioxidants or high levels of some antioxidants may harm animals they are fed to or residues remaining in animal tissues may harm humans consuming them.

Butylated hydroxyanisole (BHA) is a phenolic antioxidant that is generally recognized as safe by the FDA but its use is limited in standardized foods (CFR, 2007; part 172.110). BHA is found in cereals and confectionary products (Shahidi and Wanasundara, 1992), animal feeds and pet foods (Williams *et al*., 1999). In studies involving animals, BHA has been shown to be carcinogenic at high levels, such as 1250 ppm and higher although at lower levels (125 ppm), it may be anticarcinogenic (Williams *et al*., 1999)

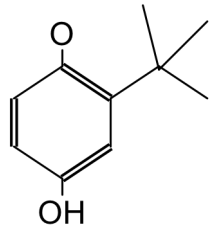

Figure 2.3 Butylated hydroxyanisole

Butylated hydroxytoluene (BHT) is a phenolic antioxidant that is generally

recognized as safe by the FDA (CFR, 2007; part 172.110). It is used in animal feeds, pet foods, cosmetics, pharmaceuticals, jet fuels, rubber and petroleum products. BHT is a fat-soluble antioxidant (Shahidi and Wanasundara, 1992) that acts as a synthetic analogue of vitamin E. BHT has been shown to be effective in reducing the rate of lipid oxidation (Wanasundara and Shahidi, 1994). There are concerns regarding the safety of including BHT in food. There have been studies involving feeding BHT at high levels to animals, which resulted in an increase in tumors within those animals. Like BHA, at low levels (100 ppm), BHT has shown anticarcinogenic properties. BHT and BHA are synergistic (Shahidi and Wanasundara, 1992), meaning they work more effectively than apart, which means less of both are needed when used in conjunction.

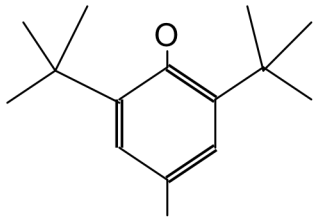

Figure 2.4 Butylated hydroxytoluene

Ethoxyquin is a phenolic antioxidant that has regulated use as a food additive. It is used in foods (CFR, 2007; part 172.140), feeds (CFR, 2007; part 573.380) and pesticides (CFR; part 180.178) as well as in spices such as chili powder and paprika to prevent colour loss (CFR, 2007; 172.140). Ethoxyquin has also been found to cause mortality in fish. The maximum level of ethoxyquin permitted in animal feed, fish food and canned pet food is 150 parts per million (CFR, 2007; part 573.380), whereas the maximum level permitted for human consumption is much lower. For example, ethoxyquin is only

permitted at 0.5 ppm in uncooked muscle meat of animals (CFR, 2007; part 172.140).

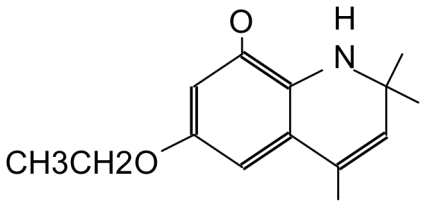

Figure 2.5 Ethoxyquin

Propyl gallate is a phenolic antioxidant commonly used in the aquaculture industry. The FDA generally regards propyl gallate as safe, although when used in human foods, its use is regulated when combined with BHA or BHT (CFR, 2007; part 172.615). Propyl gallate is added to foods containing oils and fats and chewing gum. It is also used in cosmetics, hair products, adhesives and lubricants. It does not stand up well to heat (Shahidi *et al.*, 1992), as it has a melting point of 148 ^{o}C (Shahidi and Wanasundara, 1992). There is no maximum level of propyl gallate permitted in aquafeeds by the FDA.

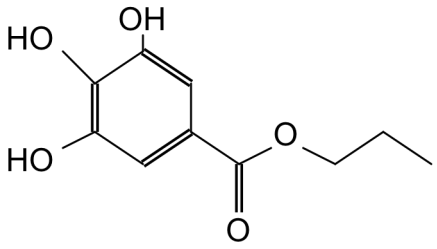

Figure 2.6 Propyl gallate

Vitamin E is the common name for eight tocopherols and tocotrienols, although

alpha-tocopherol provides the most antioxidant activity (Shahidi and Wanasundara, 1992). The FDA (2006) generally regards tocopherols as safe for use as chemical preservatives and mandates no maximum inclusion levels. However, when consumed in excess, it can be toxic (Shahidi and Wanasundara, 1992). Vitamin E reduces lipid peroxidation (Chaiyapechara *et al*., 2003; Lukaszewicz *et al*., 2004). It is also thought to improve the meat quality of fish as well as many other farmed animal species (Waagbø *et al*., 1993; Lauzurica *et al*., 2005). Vitamin E is nutritionally required in aquaculture diets (Sau *et al*., 2004) and serves as an antioxidant at levels above requirement (Waagbø *et al*., 1993).

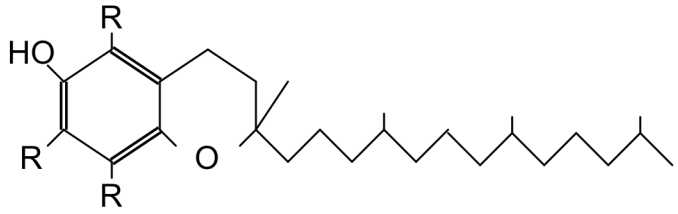

Figure 2.7 Vitamin E

Ascorbic acid, or vitamin C is an antioxidant generally recognized as safe when used responsibly (CFR, 2007; part 182.3013). Ascorbic acid works with vitamin E to reduce lipid peroxidation by returning oxidized vitamin E to its active form (Figure 2.9) (Packer *et al*., 1979). In this process, ascorbic acid is oxidized to dehydroascorbic acid, which can be reduced back to ascorbic acid by glutathione, NADP or NADPH (Deutsch, 2000).

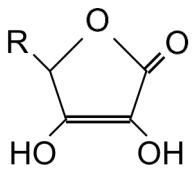

Figure 2.8 Ascorbic acid

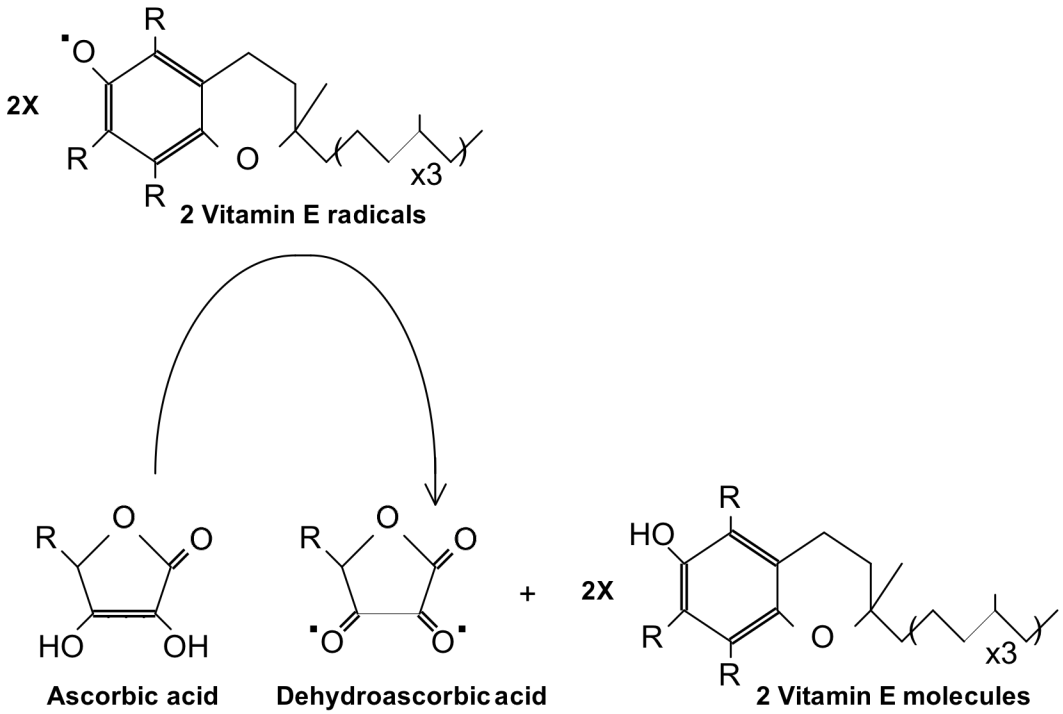

Figure 2.9 Ascorbic acid returning vitamin E to its active form. After Packer *et al*., 1979; Deutsch, 2000.

Many animal species are capable of generating vitamin C, or L-ascorbic acid *de novo* from D-glucose and do not require it in their diets. Others, such as humans and some fish species, including trout require vitamin C as an essential dietary component

(Arrigoni and De Tullio, 2002). McLaren *et al*. (1947) found the vitamin C requirements of rainbow trout can be as high as 500 mg / kg in the diet.

Vitamin C is a heat-sensitive nutrient. The efficacy of vitamin C may be compromised when exposed to improper processing conditions, such as extremes in temperatures or extended periods of exposure to heat (Laing *et al*., 1978). For protection, it may be coated with ethyl cellulose, although it is not as effective of a method of preventing loss of ascorbic acid activity as phosphorylated L-ascorbic acid (Andersen *et al*., 1998). Phosphorylated L-ascorbic acid has been found to be very effective at withstanding the extrusion process. Without any form of protection, more than half the ascorbic acid included in the mash can be destroyed during extrusion and storage (Grant *et al*., 1989).

2.10.2. Encapsulation

Encapsulation is a process that can be used to protect oils from oxidation. Saturated oil, such as palm oil (Tawfik and Huyghebaert, 1999) or other substances, such as acacia gum and hydrolyzed starches are used. This saturated oil is used to coat droplets of oil, transforming the liquid to a solid from. The encapsulation process requires three steps: emulsification of the oil with the coating material, spray drying of the emulsified product and fluid bed agglomeration (improves handling properties) of the spray dried droplets. The resulting powder is protected from the environmental oxygen and more resistant to oxidation as compared with the original oil product (Turchiuli *et al*., 2005).

The oil that is used for encapsulation may also be useful in preventing peroxidation of oils in other ways. Palm oil has been shown to be more stable than olive

and sunflower oils under stressful storage conditions (Tawfik and Huyghebaert, 1999) Palm oil also contains alpha-tocopherol, alpha-carotenes, beta-carotenes, lutein and zeaxanthin, which serve antioxidant functions (Farombi and Britton, 1999).

The oil product used for encapsulation would have an effect on the fatty acid profile of the encapsulated product, thus the diets in which they are included. In some cases, this change in the fatty acid profile may not be positive if this affects digestibility or no longer meets the fatty acid requirements of the fish. Fonseca-Madrigal *et al*. (2005) found that replacing fish oil with palm oil in the diets of rainbow did not negatively affect metabolism of lipids or fatty acids, although levels of EPA and DHA in fish tissues decreased as replacement levels of palm oil increased. Similar results were found by Bell *et al*. (2002).

2.11 Sensory analysis

Sensory analysis is used to determine a general idea of what impression a group of individuals get from a product. There are different methods to undergo sensory analysis. These various methods differ in the types of questions asked and the manner in which samples are tested. Often several different methods are used on the same samples throughout sensory analysis. Sensory analysis can be divided into difference tests, discrimination tests and preference tests. Difference tests are used to determine if there are any detectable differences between samples. Discrimination tests are used to determine what differences, if any exist. Preference tests are used to determine which of a set of samples or specific characteristics of a set of samples are favored by a group of panelists.

Several difference tests include the triangle test, the paired comparisons test and scoring. In the triangle test, three samples are presented, two of which are the same and the panelist is asked to identify the odd sample (D'Souza *et al*., 2005). In the paired comparisons test, samples are compared to determine which has a higher intensity of a specific trait than the other (Liu *et al*., 2004). When scoring is used, the intensity of a characteristic of a sample is graded using numbers or descriptive vocabulary, with the numbers generally range from 1 to 9 or 10. A low number indicates a low intensity and a high number indicates a high intensity (Regost *et al*., 2003; de Francesco *et al*., 2004). An example of descriptive vocabulary is: (1) not sweet, (2) slightly sweet and (3) very sweet.

Preference tests often include ranking and the hedonic scale. The panelist can be presented a number of samples and asked to rank them based on preference, starting with the one they prefer the most and ending on the one they prefer the least (Taylor and Walsh, 2002). The hedonic scale is used to assign a value to how much a panelist likes a sample. When analyzing fish samples, a nine- or ten-point scale is commonly used that includes descriptive terms ranging from dislike extremely to like extremely (Goulas and Kontominas, 2005; Riebroy *et al*., 2007).

2.12 Hypothesis

The replacement of fish oil with alternative ingredients is one of the central problems of aquaculture nutrition. The major problems that we must address are 1) protecting polyunsaturated vegetable oils from oxidation, 2) maintaining the fatty acid composition of fish products and 3) producing a fish product with acceptable sensory properties for consumers. Linseed oil has the highest n-3 fatty acid composition of all vegetable oils but

is extremely prone to oxidation leading to poor palatability for fish and off-flavours in fish products. Our hypothesis is that feeding linseed oil stabilized by the addition of antioxidants or by encapsulation to rainbow trout will produce fish feed with good resistance to oxidation and fish products with higher levels of EPA and DHA and acceptable sensory properties.

3. EXPERIMENTAL

3.1 Effect of stabilization of flaxseed oil with vitamin E, butylated hydroxytoluene and lipid encapsulation on quality of rainbow trout fillets.

3.2 Introduction

The world's demand for fish oil currently exceeds supplies and the aquaculture industry is still expected to grow at an annual rate of 6.5% until 2025 (Shepherd *et al*., 2005). However, replacing fish oil is problematic because of its unique fatty acid compostion. Fish oil contains high concentrations of the long chain polyunsaturated fatty acids eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) (NRC, 1993), which are required by aquaculture species for a number of functions, such as formation of anti-inflammatory prostaglandins (Yang *et al*., 2004) and leukotrienes (Kragballe *et al*., 1987).

A central goal to aquaculture is to maintain high levels of EPA and DHA in fish products is as health-conscious consumers desire high levels of these fatty acids in their diets (Schmidt *et al*., 2005). Hunter-gatherer diets are thought to have consisted of an n-3:n-6 fatty acid ratio of 1:2-3, whereas present Western diet n-3:n-6 ratios have deviated to as high as 1:10 (Cordain *et al*., 2005). Diets consisting of reduced linoleic acid (18:2n-6; LA) and higher levels of EPA and DHA have been shown to result in reduced coronary heart disease (Harris *et al*., 2007) as well as reduced risk of inflammatory diseases and depression (Kiecolt-Glaser *et al*., 2007) and improved bone health (Griel *et al*., 2007).

Most vegetable oils contain relatively low levels of n-3 fatty acids in comparison with fish oil and all are completely devoid of EPA and DHA (Bell *et al*., 2001; Geurden

et al., 2006; Rinchard *et al*., 2006). However, linseed and canola oil contains higher levels of alpha-linolenic acid (18:3n-3; ALA) than most plant-based oils as a percent of total fatty acid content, which some fish are capable of converting to EPA and DHA, with abilities varying between species (Kanazawa *et al*., 1979; Tocher *et al*., 2001; Zheng *et al*., 2004). These oils also have a higher n-3:n-6 fatty acid ratio than other vegetable oils (NRC, 1993).

Replacing fish oil with vegetable oil at high levels often results in reduced EPA and DHA concentrations in fish tissues (Bell *et al*., 2001; Caballero *et al*., 2002; Rinchard *et al*., 2006). However, action can be taken to increase the conversion of ALA to these fatty acids. Turbot have poor capabilities of synthesizing EPA and DHA (Owen *et al*., 1975), but increasing the level of ALA in the diet has been shown to improve the overall rate of conversion to EPA and DHA (Leger *et al*., 1979). Increased EPA levels in the diet can block delta-5-desaturase activity (Mozaffarian *et al*., 2005), the enzyme required to convert ALA (Buzzi *et al.*, 1997), thus preventing the conversion of ALA to EPA and DHA. Salmonids fed diets where fish oil is replaced at high levels by vegetable oil have been shown to have an increased desaturase activity, yet lower tissue EPA and DHA levels than fish fed high fish oil diets (Tocher *et al*., 2001; Tocher *et al*., 2004). The vitamin and mineral cofactors ascorbic acid, magnesium and zinc have also been shown to affect delta-6-desaturase activity, (Horrobin, 1981; Gardiner and Duncan, 1988). These findings suggest that high dietary levels of ALA, low dietary levels of EPA and proper vitamin and mineral nutrition best induce optimal conversion of ALA to EPA and DHA.

While the high content of ALA in linseed oil is beneficial nutritionally, it is also prone to oxidation during storage and processing, which may reduce its nutritional value

and decrease the conversion of ALA to EPA and DHA (Boran *et al*., 2006). It may also lead to off-flavours in fish products, reducing consumer acceptance. Antioxidants may be added to lipids to prevent their oxidation. Phenolic antioxidants, such as vitamin E and butylated hydroxytoluene (BHT) bind with free radicals and are chain-breaking antioxidants that terminate lipid peroxidation (Shahidi and Wanasundara, 1992). Vitamin E reduces lipid peroxidation (Chaiyapechara *et al*., 2003; Lukaszewicz *et al*., 2004). It serves as an antioxidant at levels above nutritional requirement and has been shown to improve the meat quality of fish (Waagbø *et al*., 1993). BHT is a fat-soluble, synthetic analogue of vitamin E (Wanasundara and Shahidi, 1994) found in products ranging from animal feeds to cosmetics and pharmaceuticals (Shahidi and Wanasundara., 1992) and is effective in reducing the rate of lipid oxidation (Wanasundara and Shahidi, 1994).

Encapsulating oils can also protect them from oxidation. Commonly substances such as palm oil, acacia gum and hydrolyzed starches are used, coating droplets of oil and protecting it from environmental oxygen (Tawfik and Huyghebaert, 1999; Turchiuli *et al*., 2005). Thus, the reduction of lipid peroxidation in vegetable oils via encapsulation or the addition of antioxidants such as vitamin E and BHT may increase the nutritional value of linseed oil in aquaculture feeds. The following experiment was performed to determine the effect of adding antioxidants to or encapsulation of linseed oil on the oxidative stability of pelleted diets, the growth of rainbow trout and the chemical and sensory properties of resulting fish fillets.

3.3 Materials and methods

3.3.1. Oil products and diets

Four products were used in these studies: 1) A commercial mixed-species fish oil (FO), 2) cold-pressed linseed oil (LO), 3) stabilized linseed oil (SLO): 946 g cold-pressed linseed oil with 20 g vitamin E and 34 g of BHT per kg and 4) encapsulated linseed oil (ELO): 350 g cold-pressed linseed oil with 7.5 g vitamin E and 12.5 g of BHT per kg of oil encapsulated in 630 g coating material primarily consisting of hydrogenated palm oil per kg. The oils were incorporated into 4 diets for the feeding experiment.

The ELO was provided by JEFO Nutrition Inc. (St. Hyacinthe, QC), the linseed oil was purchased from Bioriginal Food and Science Corp (Saskatoon, SK) and the fish oil was from EWOS Canada Ltd. (Surrey, BC). To make the SLO, the vitamin E (Lutavit® E 50) was purchased from BASF Corporation (Florham Park, NJ, USA) It is a feed grade product that is guaranteed to have 500 IU of activity per gram of product. The BHT was purchased from Sigma-Aldrich (St. Louis, MO, USA). As the ELO was purchased pre-made, the SLO was made with the same level of vitamin E and BHT per kg as the ELO product to prevent differences in experimental results due to differences in antioxidant levels.

The ingredient composition of the reference diet is shown in Table 3.1. Diets differed only in the lipid source, where the experimental oil replaced fish oil and contained 20.5 MJ / kg digestible energy and 400 g / kg of digestible crude protein (19.5 g digestible crude protein: MJ digestible energy). The diets met or exceeded all other nutrient requirements of rainbow trout (NRC, 1993). The diets used in the feeding experiment were cold extruded using a 3 mm die on a Hobart mixer. Following

extrusion, the diets were dried in a forced air oven (55 °C, 12 hours), chopped and screened to obtain a uniform pellet size.

Table 3.1 Ingredient composition of reference diet

Ingredient	Inclusion (g kg^{-1})
Corn gluten meal 60%	275.58
Wheat flour	235.54
Fish oil	178.78
Soy protein concentrate	150.00
Poultry by-product meal	108.26
Blood meal	20.00
Dicalcium phosphate	10.53
Celite	10.00
L-lysine	5.26
Choline chloride	1.00
Vitamin premix	2.50
Mineral premix	2.50
Vitamin C	0.05

3.3.2. Fish management

Triploid rainbow trout (*Oncorhynchus mykiss*) were purchased from Wild West Steelhead, Lucky Lake, SK, Canada and maintained in 360 L tanks that were part of a semi-closed recirculation system filtered biologically. The fish were fed to satiety twice daily and the amount of feed consumed by each experimental unit was recorded on a daily basis. Each tank of fish was weighed on days 0, 28, 56, 84, 112, 140 and 168. Twenty-eight tanks were used in this trial, each consisting of 22 (31 g; average weight) fish with seven replicates per treatment. Water temperature was maintained at 15 ± 2 °C. Dissolved oxygen, pH and temperature were observed and recorded daily. Chlorine,

nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light / 10 h dark cycle. The guidelines put in place by the Canadian Council on Animal Care (CCAC, 1993; CCAC, 2005) were adhered to in the maintenance of the fish.

3.3.3. Sample collection

At the end of the 168 day feeding period, one fish per tank was euthanized, gutted and frozen whole at -20 ^{o}C for fatty acid analysis. The remaining fish were euthanized and livers and viscera were weighed to determine hepatosomatic index ([wet liver weight / wet body weight] x 100) and viscerosomatic index ([wet viscera weight / wet body weight] x 100). The fish were filleted and the fillets stored at -20 °C. Finally, two left fillets were randomly selected from each tank of fish. One was vacuum-packaged and stored for 168 days at -80 ^{o}C. The other was vacuum-packaged and stored for 168 days at -20 ± 1 °C.

3.3.4. Analytical methods

3.3.4.1. Proximate analysis

Proximate analysis was used to determine the moisture (AOAC, 1990; method 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), lipid (acid ether extract) (AOAC, 1995; method 954.02), ash (AOAC, 1990; method 924.05) and protein content of all four of the experimental diets. The nitrogen content of samples was obtained using a combustion nitrogen analyzer (Leco FP-528, AOAC 1995, method 990.03). Crude protein was estimated by multiplying nitrogen content by 6.25.

3.3.4.2. Oxidative stability analysis

The Oxidative Stability Index (OSI) assay (AOCS, 1998; method cd 12b-92) was used to measure the oxidative stability of the oils and feeds used in this trial (Sun West Laboratories, Saskatoon, Saskatchewan). Oil and feed samples were stored at 18 ± 2 °C in plastic snap-top containers. Oil OSIs were measured on days 0 and 168 of the experiment while OSI measurements for the oils in the feeds were determined for the mash before pelleting, on the finished pellets immediately after pellets and on the pelleted feeds on days 0 and 168 of the experiment.

Although useful for research purposes, the pelleting method used to make the diets for this growth trial is not commonly employed. Extrusion is. To determine the effect of extrusion on the oxidative stability of the 4 diets, the diets were extruded via a co-rotating twin screw extruder (Werner and Pfeiderer, Model ZSK 57-M 50/2, Stuttgart, Germany) with a 7-hole strand die (3mm) for the fish oil diet and a one-hole strand die (5mm) for the linseed oil and stabilized linseed oil diets. The screw speed was held at 123 rpm for the fish oil diet and at 162 rpm for the linseed oil diets. The die temperature was 140 ^{o}C. The pellets were dried in a forced air oven (55 °C, 12 hours). OSI measurements were taken for the oils in the unextruded mash and the extruded pellets on day 1 following extrusion. Mash and pellets were frozen prior to OSI analysis.

3.3.5. Lipid peroxidation

The central portions of the dorsal muscles of the fillets (directly adjacent to the dorsal fin) were analyzed using the OXItek TBARS Assay Kit (Zepto Metrix Corporation, Buffalo, New York) for lipid peroxidation.

3.3.5.1. Fatty acid analysis

Fish were gutted and frozen whole at -20 ^{o}C. Muscles were later removed from the skin, fins and bones, pulled apart into pieces no larger than 1 cm^2 and thoroughly combined to produce a uniform product. A 10-15 g sample of tissue was weighed into a 250 ml Erlenmeyer flask, then homogenized with chloroform, methanol and water added at ratios of 2:2:1 using a Beckman Polytron homogenizer (Bligh and Dyer, 1959). The mixture was then filtered into a 250 ml sidearm flask using gentle suction. The Erlenmeyer flask that had contained the homogenized sample was rinsed twice with 5 ml chloroform. The filtrate was then transferred to a 250 ml separatory funnel. Several crystals of *Tert*-butylhydroquinone, 97% (TBHQ) (Sigma-Aldrich, Inc., St. Louis, MO, USA), were added to the funnel to prevent the lipids from oxidizing. The sample was left to sit overnight. The bottom chloroform / lipid layer was removed the next day into a 100 ml graduated cylinder through a funnel lined with Whatman #1 (90 mm diameter) filter paper (Whatman International Ltd., Maidstone, England) which held 1 g of anhydrous sodium sulfate (BDH Inc., Toronto, ON, Canada) to retain any water that may have passed from the separatory funnel. The volume of the chloroform layer was recorded, while the remaining methanol / water layer was discarded.

The chloroform and lipid layer was transferred from the graduated cylinder to a 250 ml round bottom flask. The lipid content of each sample was determined gravimetrically after evaporation and dessication of 5 ml samples taken from the flask. The round bottom flask was attached to a Rotary evaporator set at 50 ^{o}C. The chloroform was evaporated from the lipid as completely as possible. Using a 5 ¾" Pasteur pipette, the remaining fat was removed from the round bottom flask and transferred to a 10 ml screw-

cap test tube. A stream of nitrogen gas was directed over each fat sample to ensure the evaporation of chloroform from the sample was complete while preventing the oxidation of the fatty acids in the sample. The test tube was then flushed with nitrogen, capped and sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA) and stored at -20 ^{o}C until it was time to begin the methylation phase of the analysis. Total lipid extraction took place over several months with extraction from specific experimental units being performed randomly.

Fatty acids methyl esters of diets and fillet lipids were prepared based on the method of Keough and Kariel (1987). Following extraction of the fatty acid methyl esters, 1 ml of double distilled water was added to the sample and vortexed. 1.5 ml of hexane was then added and vortexed. The hexane layer was removed and placed in a test tube. Twice, 1.5 ml of hexane was added to the sample, vortexed, removed and placed in the test tube. 1.5 ml of double distilled water was added to the test tube and slowly mixed. The water layer was removed and discarded. Another 1.5 ml of double distilled water was added and slowly mixed.

Several crystals (approx. 0.1-0.2 g) of TBHQ were added to a screw-cap test tube. The hexane layer was removed from the test tube to a screw-cap test tube, and heated at 40 ^{o}C under a stream of nitrogen until the hexane completely evaporated. The remaining fatty acid methyl esters in the test tube were dissolved with 1 ml hexane. The tube was vortexed and the sample was filtered through a glass syringe with a 0.45 um filter into a gas chromatography vial. The vial was flushed with nitrogen, capped, wrapped with Parafilm® and stored at -20 ^{o}C until ready for analysis.

Fatty acid analysis was performed via gas chromatography (GC) on an Agilent

6890 system (Hewlett Packard 6890 GC, Rev A.03.07, Agilent Technologies, Inc. Santa Clara, CA, USA). The column was a Fused Silica Capillary Column SP 2560. The gases used for this experiment consisted of hydrogen, air and helium, with helium being the carrier gas. A stock solution of a gas chromatography standard (purchased from Nu-Chek Prep, Inc., Elysian, MN, USA) was created, with hexane as the solvent. The percentage of all fatty acids present in the standard was known. The temperature program had an initial oven temperature of 140 ^{o}C, which was raised at a rate of 6 ^{o}C per minute to the final oven temperature of 240 ^{o}C, which was held for a period of 20 minutes with total run time being 41.67 minutes. Methyl esters were identified by comparison with the known standards based on run time. The area within each individual peak was compared with the total area of all the peaks of a sample to show each fatty acid as a percentage of the sample's total fatty acids.

3.3.5.2. Sensory analysis

An untrained, consumer taste panel consisting of 43 members and a trained taste panel consisting of 12 members analyzed the fillets for their sensory attributes. All panelists involved in this experiment were non-smokers, excluding one trained panelist who smoked occasionally. There were 20 male and 23 female consumer panelists. Of the consumer panelists, 23 were between the ages of 18 and 25; 12 were between the ages of 26 and 34; the remaining 8 were between the ages of 35 and 65. All of the consumer panelists regularly ate fish. Forty percent of them ate fish once a month or less, the remaining 60% ate fish more frequently. The predominant types of fish eaten by the consumer panelists were salmon (88% of consumer panelists), tuna (61%), shellfish

(40%), trout (35%) and whitefish (35%). There were 11 female and 1 male trained panelists. Eight of the trained panelists were between the ages of 18 and 25; 2 were between the ages of 26 and 34; the remaining 2 were between the ages of 35 and 54. The trained panelists ate fish regularly. Twenty-five percent of the panelists ate fish once a month or less; 75% ate fish on a more frequent basis. The types of fish mainly eaten by the trained panelists include salmon (92%), tuna (58%) shellfish (58%) and whitefish (33%).

Prior to sensory testing, approval was requested from and granted by the University of Saskatchewan Behavioural Research Ethics Board (Saskatoon, SK, Canada; BEH# 06-22) to ensure the testing methods and questioning met ethical requirements put in place by this organization for the health and safety of the participants volunteering to take part in this study. All panelists were asked to ensure they had no allergies to fish, fish oil, linseed oil, flax or any type of seafood. They were instructed to inform the researchers immediately if they experienced any allergic reactions so that appropriate help could be sought out immediately. Panelists were asked to refrain from smoking, chewing gum, drinking (excluding water) or eating 1 hour prior to conducting sensory analysis. Prior to sensory analysis, both untrained and trained panelists were asked their gender, whether or not were smokers, their age group, how often they ate fish and which types, if any that they ate.

The panels were conducted in the food and sensory evaluation facility located in the Agriculture Building at the University of Saskatchewan. The facility consisted of two adjoining rooms: a kitchen where samples were prepared and cooked and a sensory testing room, where sensory testing took place. The sensory testing room contained seven

individual booths, each of which had a pass-through sample presentation door in front, through which samples were passed from the kitchen to the panelists once they were seated without permitting any contact between panelists and sensory staff. This arrangement prevented panelists from seeing into the kitchen, thus receiving no hints as to how samples were prepared nor the identity or labeling of samples. Red lighting was used to prevent panelists from making unnecessary judgments about other attributes of the samples based on their appearance.

Samples were assigned random three-digit numbers and the order in which they were presented to each panelist was also randomly determined. Samples (approx. 1.5cm^3) were wrapped in heavy-duty aluminum foil and steamed for 15 minutes to ensure their temperature was above 72 ^oC (which is necessary for health reasons). They were kept warm by being placed in a warming oven set at 60 ^oC.

The fish samples remained in the oven until the panelists arrived. The samples were then presented to each panelist on an individual ceramic tile (also warmed to 60 ^oC) wrapped in heavy-duty aluminum foil. This was placed on a tray that also included a glass of water, a glass of lemon water (labeled), three unsalted soda crackers on a Petri dish, a fork on a paper napkin and a pencil. The water and crackers were provided for each panelist to cleanse their palates. Panelists were asked to take a bite of cracker and a drink of water before beginning and between each sample. The lemon water was provided as an optional method of cleansing the palate. If a panelist chose to use the lemon water, they were asked to do so between each sample to ensure the results they provided were consistent.

Panelists were instructed to tear open the aluminum foil packet and smell the fish

sample to assess its aroma. They were then instructed to take a bite of fish and assess the remaining sensory attributes. They were asked to ensure to leave a second bite in case they required one to help them decide on their scoring.

The untrained taste panel was used to assess consumer preference. Panelists were asked to state their perception of the intensity of flavour, aroma, juiciness and texture and how much they did or did not like the specific attributes of each fish sample. The purpose of the trained panel was to identify differences that may have existed between specific attributes of the fish such as flavour, aroma, juiciness and texture. Red lighting was used to prevent any biases based on differences in colour between samples.

The trained panelists were trained over seven training sessions (Appendix 2) and spent another seven sessions analyzing the fillets from this experiment. Each analysis, the panelists were presented with fillets from each of the four treatments. For each session, origin of the fillets was a different tank than one analyzed in a previous session. In this way, all experimental units were analyzed separately from one another.

3.3.6. Colour analysis

Colour analysis was determined using a Hunterlab miniscan XE (Reston, Virginia, USA). Two fillets per tank were analyzed for L* (lightness) a* (redness) and b* (yellowness) values. Two measurements were taken from each fillet on the inner portion of the fillet (skin side away from instrument port) with the first value taken from the portion of fillet directly below the dorsal fin and the second value taken by rotating the fillet 90^{o}. These two values were then averaged for each fillet.

3.3.7. Statistical analysis

No statistical analyses were done on oxidative stability indices. Only one assay per sample was done due to the high cost of the OSI analysis. The other experiments were analyzed as a completely randomized design with 4 treatments. The analysis of TBARS in fish fillets was analyzed as a 2 x 4 factorial design with 2 sampling times and 4 oil products. Analyses of all but the sensory results were performed using the General Linear Model procedure of SPSS (2005; version 14.0). Analysis of sensory results used the Mixed Models procedure of SAS (2004; version 9.1) The Ryan-Einot-Gabriel-Welsh F test was used to determine differences between means. Results were considered significant when $P < 0.05$.

3.4 Results

3.4.1. Growth trial

There were no significant differences ($P > 0.05$) in any of the growth performance parameters during the growth trial due to diet composition (Table 3.2). The hepatosomatic and viscerosomatic indices are measures of nutritional stress in the fish and these parameters were not significantly affected by the replacement of fish oil with any of the linseed oil products. These results are not surprising, as nutritionally, the diets were almost identical with the only differences between the diets being in the fatty acid composition of the oil sources and the presence or absence of antioxidants. Mortality was significantly lower in the fish fed the SLO diet (12.34%) compared to the ELO diet (24.68%) while the FO (18.18%) and LO (20.78%) diets were intermediate in the levels of mortality.

Table 3.2 Growth performance and mortality of rainbow trout fed fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO) diets during a 168 day feeding period

Parameter	FO	LO	SLO	ELO	SEM
Weight					
Initial(g)	31.1	30.2	31.0	32.8	0.63
Final (g)	109.9	113.5	106.7	112.0	5.16
Gain (g)	78.8	83.3	75.7	79.3	5.13
Gain (%)	205.4	215.5	199.8	184.2	17.44
SGR (% / d)	1.0	1.0	1.0	0.9	0.06
Feed intake (g / d)	115.9	107.7	112.1	105.2	5.02
FCR (g feed / g gain)	1.5	1.4	1.4	1.4	0.09
PER (g protein / g gain)	2.0	2.2	1.9	2.1	0.14
HSI (%)	1.1	1.1	1.0	1.1	0.06
VSI (%)	83.3	84.5	83.3	80.7	1.23
Mortality (%)	18.2^{ab}	20.8^{ab}	12.3^{a}	24.7^{b}	0.60

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM=Standard error of the mean.
SGR=Specific growth rate ([ln final weight – ln initial weight] / time (days) x 100).
FCR=Feed conversion ratio (feed intake / wet weight gain).
PER=Protein efficiency ratio (wet weight gain / protein intake).
HSI=Hepatosomatic index ([wet liver weight / wet body weight] x 100).
VSI=Viscerosomatic index ([wet viscera weight / wet body weight] x 100).

3.4.2. Oxidation of oil products and diets

The oxidative stability indices (OSI) of the four oils and the prepared diets on days 0 and 168 are shown in Table 3.3. The OSI indicates the length of time to completely oxidize a sample under controlled conditions. Thus, a higher OSI indicates greater resistance to oxidation. The OSI of the FO (1.04 h) and LO (1.78 h) were low compared with the SLO (10.13 h) and ELO (18.40 h) at the beginning of the experiment. The OSI of the LO was reduced to 0 h after the 168 day storage period while the SLO and ELO maintained high OSI values throughout the experiment. Similar trends were seen with the diets. The OSIs

of the FO and LO diets were low and were reduced to 0 after 168 days of storage while the SLO and ELO diets maintained high OSIs throughout the trial. Extrusion of the feeds had a marked effect on OSI (Table 3.4). In all cases, the OSIs of the products were greatly reduced after extrusion. However, the SLO product had a markedly higher OSI of 3.35 h after extrusion compared to 0.30 and 0.86 h for the FO and LO diets after extrusion.

Table 3.3 Oxidative stability index (in hours) of fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO) in raw and diet form after 0 and 168 days storage at room temperature

	Storage time (days)	
Product	**0**	**168**
Oils		
FO	1.04	0.75
LO	1.78	0.00
SLO	10.13	9.20
ELO	18.40	25.20
Diets		
FO	0.52	0.00
LO	0.91	0.00
SLO	6.70	6.40
ELO	13.80	11.40

Table 3.4 Oxidative stability index (in hours) of diets containing fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO) in mash form and following extrusion

Product	Before extrusion	After extrusion
FO	2.24	0.30
LO	1.26	0.86
SLO	6.20	3.35
ELO	n.d.	n.d.

3.4.3. Composition of rainbow trout fillets

The levels of thiobarbituric acid reactive substances (TBARS), more specifically malonaldehyde (MDA) in the fillets of the fish fed the 4 different oil sources after 0 or 168 days of storage are shown in Table 3.5. There was no significant effect of oil product on the levels of TBARS in fillets (P = 0.20). However, there was a trend of lower TBARS levels in fish fed the SLO diet compared to the LO diet. The effect of storage on the level of TBARS in fillets was significant (P = 0.02) with the fillet TBARS increasing from 0.11 umol MDA / g tissue on day 0 to 0.18 umol MDA / g tissue. There were no significant interactions between diet and storage treatments.

Table 3.5 Thiobarbituric acid reactive substances (TBARS) found in fillets of rainbow trout fed experimental diets following two different storage temperatures

	umol MDA / g tissue	umol MDA / g fat in tissue
Oil product		
Fish	0.12	2.72
Linseed	0.17	4.00
Stabilized linseed	0.13	2.89
Encapsulated linseed	0.16	4.44
SEM	0.027	0.776
Storage temperature		
-80 ^{o}C	0.11[a]	2.67[a]
-30 ^{o}C	0.18[b]	4.35[b]
SEM	0.019	0.549
***P* value**		
Oil product	0.20	0.09
Storage temperature	0.02	<0.01
Interaction	0.59	0.79

[a,b,c] Means in the same column with different superscripts are significantly different ($P < 0.05$).
MDA=Malonaldehyde
SEM=Standard error of the mean.

Table 3.8 shows lipid content of fillets of fish fed the ELO diet to be significantly higher than fish fed the other 3 diets ($P < 0.05$), whereas the ELO diet was found to be lower in lipid content (24.6%) than the other three diets (28.1, 28.3, 27.0 %, for FO, LO and SLO diets, respectively) (Table 3.7). Although the experimental oils are higher in levels of 18:3n-3 than the fish oil control, they are completely devoid of 20:5n-3 and 22:6n-3 (Table 3.6). Table 3.6 shows the ELO diet is also much higher in levels of 16:0 (39.3%) than the others (21.7, 7.5 and 7.1% for FO, LO and SLO treatments,

respectively).

The fatty acid composition of the fillets is shown in Table 3.8. Tissue 18:3n-3 levels of the LO-fed fish were significantly higher than those of the FO and ELO-fed fish ($P < 0.05$). 20:5n-3 and 22:6n-3 levels in fish fed the FO treatment were highest and those of fish fed the LO treatment are lowest, although there are no significant differences between treatments ($P > 0.05$). Fish fed the ELO diet had significantly higher levels of 16:0 than the LO treatment, although fish fed the FO diet had the highest saturate levels, which were also significantly higher than those of the fish fed the LO diet.

Table 3.6 Lipid (%, as is) and major fatty acid composition (% of total fatty acids) of fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO)

	FO	LO	SLO	ELO
Lipid content	95.7	96.8	95.1	75.9
Saturates				
14:0	5.4	0.0	0.7	0.8
15:0	0.8	0.0	0.0	0.0
16:0	18.5	5.0	4.9	32.7
17:0	0.6	0.0	0.0	0.0
18:0	2.7	3.4	3.3	17.5
Total saturates	31.7	8.7	9.4	51.4
Monoenes[1]				
16:1 cis	6.1	0.1	0.0	0.0
18:1 cis	17.3	16.2	16.0	8.9
20:1 cis	0.3	0.0	0.0	0.0
22:1 cis	0.4	0.0	0.1	0.0
24:1 cis	0.1	0.1	0.1	0.0
Total monoenes	28.1	16.5	16.3	9.5
n-6 Polyunsaturates				
18:2n-6 *cis*	2.7	16.3	16.0	8.1
20:3n-6	4.3	0.0	0.0	0.0
20:4n-6	0.3	0.0	0.0	0.0
22:2n-6	0.7	0.0	0.1	0.0
Total n-6 PUFA	8.4	16.5	16.4	9.0
n-3 Polyunsaturates				
18:3n-3	2.9	58.3	57.5	28.3
20:5n-3	9.6	0.0	0.0	0.0
22:6n-3	12.9	0.0	0.0	0.0
Total n-3 PUFA	25.4	58.3	57.5	28.3
Total PUFA	33.8	74.8	73.9	37.4
n-3:n-6	3.0	3.5	3.5	3.1

Values are as % of total fatty acids.
[1]Monoene isomers were pooled.
PUFA=Polyunsaturated fatty acids.

Table 3.7 Lipid (%, as is) and major fatty acid composition (% of total fatty acids) of experimental diets made from fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO)

	FO	LO	SLO	ELO
Lipid content	28.1	28.3	27.0	26.4
Saturates				
14:0	5.2	0.1	0.1	0.5
15:0	0.7	0.0	0.0	0.0
16:0	21.7	7.5	7.1	39.3
17:0	0.6	0.1	0.1	0.0
18:0	3.5	3.8	3.6	23.6
Total saturates	34.2	11.7	11.3	63.4
Monoenes[1]				
16:1 cis	6.6	0.6	0.6	0.5
18:1 cis	23.5	20.5	19.1	10.3
20:1 cis	0.4	0.0	0.0	0.0
22:1 cis	0.4	0.1	0.1	0.0
24:1 cis	0.0	0.0	0.1	0.0
Total monoenes	34.9	21.7	20.2	10.9
n-6 Polyunsaturates				
18:2n-6 *cis*	7.1	19.1	18.6	11.0
20:3n-6	4.4	0.0	0.0	0.0
20:4n-6	0.1	0.0	0.0	0.0
22:2n-6	0.4	0.0	0.0	0.0
Total n-6 PUFA	12.3	19.3	18.8	11.2
n-3 Polyunsaturates				
18:3n-3	1.8	47.3	49.2	14.3
20:5n-3	5.2	0.0	0.0	0.0
22:6n-3	6.7	0.0	0.0	0.0
Total n-3 PUFA	13.8	47.3	49.2	14.3
Total PUFA	26.1	66.6	68.1	25.5
n-3:n-6	1.1	2.5	2.6	1.3

Values are as % of total fatty acids.
[1]Monoene isomers were pooled.
PUFA=Polyunsaturated fatty acids.

Table 3.8 Lipid (%, as is) and major fatty acid composition (% of total fatty acids) of lipid from muscle tissue of rainbow trout fed fish oil (FO), linseed oil (LO), stabilized linseed oil (SLO) and encapsulated linseed oil (ELO)

	FO	LO	SLO	ELO	SEM[1]
Lipid content	3.8[a]	4.4[a]	4.2[a]	5.4[b]	0.27
Saturates					
14:0	1.5[ab]	0.7[a]	1.2[ab]	2.2[b]	0.34
15:0	0.3	0.1	0.2	0.3	0.05
16:0	15.5[b]	11.0[a]	13.8[ab]	14.8[b]	0.94
17:0	0.1	0.1	0.1	0.2	0.04
18:0	4.5[b]	3.9[ab]	4.4[b]	3.5[a]	0.24
Total saturates	26.1[b]	22.4[a]	24.7[ab]	25.1[ab]	0.83
Monoenes[2]					
16:1 cis	3.3[ab]	1.5[a]	2.7[ab]	3.9[b]	0.52
18:1 cis	22.7[b]	19.1[a]	21.4[b]	20.9[ab]	0.00
20:1 cis	0.2	0.2	0.2	0.3	0.08
22:1 cis	0.5	0.9	0.7	0.6	0.13
24:1 cis	0.3	0.2	0.3	0.4	0.53
Total monoenes	30.1[b]	23.6[a]	27.6[b]	28.1[b]	1.06
n-6 Polyunsaturates					
18:2n-6 *cis*	11.3	14.2	12.5	10.5	1.05
20:3n-6	0.5	0.1	0.1	0.8	0.22
20:4n-6	0.2	0.3	0.2	0.4	0.11
22:2n-6	1.0[a]	1.7[b]	1.1[a]	1.1[ab]	0.17
Total n-6 PUFA	13.8	16.8	14.6	13.2	1.03
n-3 Polyunsaturates					
18:3n-3	12.6[a]	26.5[b]	17.9[ab]	12.6 [a]	3.55
20:5n-3	3.5	2.8	3.2	4.4	0.08
22:6n-3	11.0	6.1	9.0	11.8	1.99
Total n-3 PUFA	27.6[a]	35.5[b]	30.2[ab]	28.9[a]	1.66
Total PUFA	41.4[a]	52.4[b]	44.9[ab]	42.1[a]	2.26
n-3:n-6	2.1	2.1	2.1	2.2	0.16

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM=Standard error of the mean.
Values are as % of total fatty acids.
[1]Pooled standard error of the mean.
[2]Monoene isomers were pooled.
PUFA=Polyunsaturated fatty acids.

3.4.4. Sensory Panels

The trained panelists found the FO treatment to have a significantly more intense aroma intensity than the SLO treatment ($P < 0.05$), which was mostly attributed to rancidity (Table 3.9). They also found the aroma desirability of the SLO treatment to be significantly more desirable than that of the FO treatment ($P < 0.05$) (Table 3.10). Table 3.9 shows the FO treatment also had a significantly higher rancid flavour intensity than the other three treatments, but a significantly lower earthy / musty flavour intensity than the ELO ($P < 0.05$). The SLO and ELO treatments had a significantly higher corn aroma than the fish oil treatment ($P < 0.05$). There were no significant differences between treatments regarding flavour desirability, firmness and juiciness (Table 3.10). The trained panelists found the SLO and ELO treatments to be significantly more acceptable on an overall basis than the fish oil treatment ($P < 0.05$).

Results for differences between treatments as determined by consumer panelists are shown in Table 3.11. There were no significant difference between treatments for aroma and flavour intensity, nor were there any significant differences in texture and juiciness ($P > 0.05$). The consumer panelists also established there were no significant differences between treatments on the acceptability of flavour, aroma, texture and juiciness and determined there were no significant differences between the overall acceptability of the treatments ($P > 0.05$) (Table 3.12). When asked if they would purchase the fish products in the future (Table 3.13), the consumer panelists did not vary significantly in their responses of probably yes for the four treatments ($P > 0.05$).

Table 3.9 Aroma and flavour intensities of fillets of rainbow trout fed experimental diets as determined by trained sensory panelists

	Treatment				SEM
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	
Aroma	6.1[b]	5.8[ab]	5.5[a]	5.7[ab]	0.11
Fish aroma	3.4	2.8	2.7	2.7	0.20
Paint aroma	0.7	0.7	0.8	0.3	0.18
Rancid aroma	1.3[b]	0.6[a]	0.7[a]	0.6[a]	0.17
Earthy / musty aroma	1.1[ab]	1.2[ab]	0.8[a]	1.5[b]	0.15
Corn aroma	0.2[a]	0.6[ab]	0.7[b]	0.6[b]	0.12
Nutty aroma	0.3[a]	0.9[b]	0.6[ab]	0.6[ab]	0.13
Flavour intensity	5.5	5.4	5.1	5.0	0.13
Fish flavour intensity	3.1	2.2	3.0	2.3	0.12
Paint flavour intensity	0.6	0.7	0.5	0.2	0.14
Rancid flavour intensity	1.21[b]	0.5[a]	0.6[a]	0.3[a]	0.14
Earthy / musty flavour	1.0[a]	1.5[ab]	1.2[ab]	1.6[b]	0.15
Corn flavour	0.2	0.4	0.4	0.4	0.11
Nutty flavour	0.3	0.6	0.5	0.5	0.11

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

SEM=Standard error of the mean.

1 - Extremely mild.
2 - Very mild.
3 - Moderately mild.
4 - Slightly mild.
5 - Slightly intense.
6 - Moderately intense.
7 - Very intense.
8 - Extremely intense.

Table 3.10 Evaluation by trained sensory panelists on aroma desirability, flavour desirability, firmness and juiciness of fillets of rainbow trout fed experimental diets and overall acceptability

	Treatment				
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	SEM
Aroma desirability	4.3[a]	4.6[ab]	4.9[b]	4.7[ab]	0.13
Flavour desirability	4.6	4.4	4.6	4.8	0.22
Firmness	4.8	4.9	4.7	4.8	0.10
Juiciness	4.9	4.9	5.0	4.8	0.10
Overall acceptability	4.4[a]	4.7[ab]	4.9[b]	5.0[b]	0.13

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

SEM=Standard error of the mean.

1 - Extremely undesirable, soft, dry.
2 - Very undesirable, soft, dry.
3 - Moderately undesirable, soft, dry.
4 - Slightly undesirable, soft, dry.
5 - Slightly desirable, firm, juicy.
6 - Moderately desirable, firm, juicy.
7 - Very desirable, firm, juicy.
8 - Extremely desirable, firm, juicy.

Table 3.11 Sensory evaluation by consumer panelists on attributes of fillets of rainbow trout fed experimental diets

	Treatment				SEM
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	
Aroma	3.6	3.1	3.0	3.2	0.18
Flavour	3.3	3.1	3.1	3.2	0.15
Texture	3.0	3.1	3.4	3.0	0.12
Juiciness	3.7	3.7	3.8	3.7	0.14

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

SEM=Standard error of the mean.

1 - Very mild, bland, firm, dry.
2 - Moderately mild, bland, firm dry.
3 - Slightly mild, bland, firm, dry.
4 - Slightly strong, intense, soft, juicy.
5 - Moderately strong, intense, soft, juicy.
6 - Very strong, intense, soft, juicy.

Table 3.12 Acceptability of sensory attributes of fillets of rainbow trout fed experimental diets as determined by consumer panelists

	Treatment				SEM
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	
Aroma	3.8	4.2	4.2	3.9	0.17
Flavour	3.9	4.4	4.2	4.0	0.16
Texture	4.5	4.5	4.6	4.3	0.12
Juiciness	4.3	4.3	4.3	4.4	0.15
Overall	4.0	4.3	4.1	4.0	0.16

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

SEM=Standard error of the mean.

1 - Dislike very much.
2 - Dislike moderately.
3 - Dislike slightly.
4 - Like slightly.
5 - Like moderately.
6 - Like very much.

Table 3.13 Likelihood of consumer panel to purchase fillets of rainbow trout fed experimental diets

	Treatment				
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	SEM
Is this a product you might purchase in the future?	2.5	2.8	2.8	2.6	0.11

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM=Standard error of the mean.
1 - Definitely no.
2 - Probably no.
3 - Probably yes.
4 - Definitely yes.

3.4.5. Colour analysis

As shown in Table 3.14, there is no significant ($P<0.05$) difference existing between the four treatments for lightness (L*). However, for both redness (a*) and yellowness (b*) values, the fish oil treatment has significantly higher values than the other three treatments with values of 3.59 for redness and 25.35 for yellowness.

Table 3.14 Evaluation of fillets of rainbow trout fed experimental diets for lightness (L*), redness (a*) and yellowness (b*)

	Treatment				
	Fish	Linseed	Stabilized linseed	Encapsulated linseed	SEM
L*	69.45	72.31	68.28	70.5	1.473
a*	3.59[b]	2.07[a]	1.86[a]	2.03[a]	0.435
b*	25.35[b]	21.22[a]	20.80[a]	20.51[a]	0.774

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).

SEM=Standard error of the mean

L*=Lightness

a*=Redness

b*=Yellowness

4. DISCUSSION

Addition of antioxidants and the use of encapsulation in this experiment were determined to increase the storage time of linseed oil. Stabilization of linseed oil was also found to decrease its susceptibility to peroxidation during extrusion. Wagner and Elmadfa (2000) investigated the addition of alpha-tocopherol to linseed oil and found adding 1 g alpha-tocopherol / kg linseed oil resulted in a significant increase in OSI. At zero antioxidant inclusion, the OSI of linseed oil was 1.13 hours while at 1 g / kg, its OSI was 1.18 hours. This was a minor increase in oxidative stability as compared with those found in this experiment (SLO's OSI was 8.35 hours longer than LO's OSI). However, the amounts of antioxidants added in the present study were also higher than that of Wagner and Elmadfa (2000). Bautista-Teruel and Subosa (1999) investigated the addition of BHT to shrimp diets at a level of 0.5 g/ k g and found it reduced lipid oxidation of the feed when stored at 10 ^{o}C as well as resulting in higher shrimp growth and survival rates. The results of this experiment suggest that addition of both vitamin E and BHT to aquafeeds may be useful, as both serve as antioxidants and the addition of both allows for a higher level of antioxidant activity in the diet without one of the compounds being included at levels that could result in toxicity.

Growth of the fish was not affected by the experimental treatments. Other studies have also reported replacing fish oil with vegetable oil does not significantly affect growth performance of rainbow trout (Arzel *et al*., 1994; Bell *et al*., 2002; Caballero *et al*., 2002; Tocher *et al*., 2003). There were no significant differences between treatments on the MDA levels of the fillets, which shows all four oil treatments produced equally

non-rancid fillets that would be considered equally healthy in terms of oxidative stability. Furthermore, the interaction between oil treatments and storage temperatures was not significant, which showed the four treatments were not affected differently by storage conditions. Although the fillet MDA levels did not differ according to diet, the addition of vitamin E has been shown to improve the meat quality of fish (Waagbø *et al*., 1993), which may have affected the results obtained by the taste panelists.

There were few significant differences found between the treatments for sensory attributes. Consumer panelists were unable to detect any differences between the treatments and had no preferences for a specific treatment, whereas trained panelists found fish fed the experimental diets to be similar to or more acceptable than fillets of fish fed the FO treatment. Drobna *et al*. (2006) reported that fish fed diets containing linseed oil had significantly reduced odours, and oily tastes compared to fish fed a fish oil control diet. In turbot, Regost *et al*. (2003) reported feeding linseed oil resulted in a decrease in odour intensity and marine flavour compared to fish fed fish oil. Lastly, de Francesco *et al*. (2004) reported that panelists consuming rainbow trout that had fishmeal replaced with plant proteins found significantly lowered levels of flavours and odours in the plant protein fed fish. Since fishmeal contains a significant amount of fish oil, this may have been due to a decrease in the amount of fish oil in the diet. Taken together, these results suggest that replacement of fish oil with linseed oil will result in detectable differences in the flavour and odour of fish but that these differences are relatively small and will be acceptable to most consumers.

Dietary fatty acids directly influence tissue fatty acids in rainbow trout (Fonseca-Madrigal *et al*., 2005). The EPA and DHA in the fish oil diet was likely the major

determining factor on the presence of these fatty acids in fish fed this diet, rather than fatty acid bioconversion as the percentage of ALA was only 1.8% of total fatty acids. The EPA and DHA levels of the fish fed the LO oil treatment were numerically but not significantly lower than those fed the FO diet, indicating bioconversion of ALA to EPA and DHA, as these PUFAs were not supplied in the diet. Other studies reported significantly lower levels of EPA and DHA in fish fed vegetable oil (Bell *et al*., 2001; Bell *et al*., 2002; Bell *et al*., 2004; Rinchard *et al*. 2006). The ALA levels of the LO and SLO diets were similar (47.3 and 49.2% of total fatty acids, respectively). While no similar studies have been performed in rainbow trout, Zanini *et al*. (2004) reported the addition of 400 mg / kg vitamin E to linseed oil-fed to chickens resulted in significant increases in ALA and DHA in breast meat although the level of EPA was not affected by vitamin E addition.

Although referred to as ELO, the ELO only consisted of 35% linseed oil, with 2% consisting of antioxidants and the remainder (63%) being palm oil. This high palm oil composition resulted in a diet that was high in the saturated fatty acid 16:0, or palmitic acid, which is a predominant fatty acid found in palm oil and low in ALA due to the low linseed oil composition. Results from Bell *et al*. (2002) show that as fish oil was replaced with crude palm oil at increasing levels in Atlantic salmon diets, the palmitic acid composition of the diets increased and the ALA composition of the diets decreased. Fish fed the ELO diet had similar tissue EPA and DHA levels than the LO-fed fish, despite the diet being lower in ALA than the LO diet (14.3% vs 47.3% of total fatty acids). The ELO diet had the highest OSI, which suggests the prevention of oxidation of linseed oil increases the bioconversion of ALA to EPA and DHA. The mechanism may be due to an

increase in non-oxidized ALA being available for conversion in the ELO diet compared to the LO diet, resulting in similar EPA and DHA levels.

The ELO-fed fish had a significantly higher body fat percentage than fish fed the other two treatments. As humans wish to consume higher levels of EPA and DHA, although two fillets of fish may have similar EPA and DHA levels as a percent of total fatty acids, the fillet that has a higher fat content will deliver a higher volume of these fatty acids than a fillet with a lower fat content. The increased body fat percentage of the ELO-fed fish may be due to the higher saturated fatty acid content of the ELO diet than the others, as other research has found that an increase in saturated fatty acid content can result in an increase of body fat accretion. Kishino *et al*., found a positive correlation in overweight human males between serum levels of palmitic acid (16:0) and other saturated fatty acids and visceral fat thickness. There were no significant differences between treatments regarding the viscerosomatic indicies of fish fed the four treatments, yet there was still an increase in percent body fat.

There was no identifiable cause of the mortality seen in these fish. As there were only 22 fish per tank, the mortality result are an average of 4.0, 4.6, 2.7 and 5.4 fish dying per tank for the FO, LO, SLO and ELO treatments, respectively, over the entirety of the 168 day trial. This mortality rate may have been related to diet, or may have been due to natural or unrelated causes. If the mortality rate was caused by the diets, there are two interesting points. Firstly, the high levels of saturated fats in the diet may have caused the significantly higher ($P < 0.05$) increase in mortality for the ELO-fed fish. Human research indicates that high levels of saturated fats in the diet may increase the risk of coronary

heart disease, especially palmitic acid and myristic acid (14:0), both of which are found in palm oil (Sidani and Ziegler, 2008). An even more intriguing set of results is the significantly lower ($P < 0.05$) mortality rate for the SLO-fed fish than for all of the other treatments. There may have been a health benefit associated with the addition of the vitamin E and BHT that reduced the rate of mortality below that of the fish consuming the FO diet, although similar results may have been seen if these antioxidants had been added to the FO diet.

The diets used in the present experiment were completely lacking fishmeal. Thus, no fish oil and no EPA or DHA was supplied in the linseed oil diets. Fatty acid biosynthesis has been shown to occur at higher rates in diets lacking EPA, as EPA in the diet blocks delta-5-desaturase activity (Mozaffarian *et al*., 2005). As the experimental diets were completely lacking EPA, delta-5-desaturase activity may have been upregulated in the present experiment.

The diets in the present trial were pelleted using a cold extruder as opposed to being processed by extrusion. Vitamin C is a heat-labile feed ingredient (Laing *et al*., 1978) responsible for recycling oxidized vitamin E to its active form (Packer *et al*., 1979). It has also been found to perform as a cofactor in fatty acid biosynthesis by increasing delta-6-desaturase activity (Gardiner and Duncan, 1988), the enzyme considered to be rate-limiting in fatty acid biosynthesis (Brenner, 1981). As the diets were cold extruded, the likelihood of the vitamin C being destroyed in the process decreased, and there may have been more available for the bioconversion of ALA to EPA and DHA.

The replacement of fish oil with vegetable oils is necessary to sustain the growth

of aquaculture. Replacing fish oil with a vegetable-based oil containing high levels of ALA and the use of antioxidants to preserve the quality of these highly unsaturated oils appears to be a viable strategy to achieve a desirable fatty acid composition in rainbow trout and meet consumer expectations for flavour and nutrient composition.

5. CONCLUSION

There is growing recognition that the levels of omega-3 fatty acids need to be increased in Western diets. Increasing the amount of salmonid fish in diets is seen as one of the most promising ways of achieving this goal. Salmon and trout reared in aquaculture already account for over 50% of salmonid products so it is essential that the nutritional properties of farmed fish be maintained. As fish oil supplies become inadequate for the feeding of the rapidly growing aquafeed industry, we must develop strategies to increase the use of vegetable oils in salmonid diets.

There are presently three approaches to the problem. Firstly, the development of GMO varieties of oilseed crops that produce EPA and DHA is well underway. Like fish oil, these GMO oils will contain EPA and DHA and supply fish with adequate dietary levels of these long-chain omega-3 fatty acids. Already, canola seeds have been modified to produce stearadonic acid (Ursin, 2003). This approach could potentially offer another source of EPA and DHA for both human and aquaculture nutrition. The downside of this approach is that the many of the major producers of salmon in the world will not use GMO feed ingredients. Europe, Japan and Chile all have bans in place on the use of GMO ingredients and this does not appear likely to change in the short term. However, in the long term, this approach will create large, sustainable sources of highly-unsaturated fatty acids for aquaculture.

A second approach to this problem is feeding vegetable oils in the early growth periods followed by feeding fish oil during the finishing period. This approach can result

in a recovered fatty acid profile in some fish, as opposed to that of fish fed only vegetable oil. Izquierdo *et al*. (2005) found late feeding of fish oil to vegetable oil-fed gilthead seabream resulted in the recovery of DHA, but not EPA. These practices are an improvement on reducing the pressure on existing fish oil stocks and could prove useful in extending the availability of fish oil further into the future. However, they still depend on the use of fish oil, albeit at lower levels, and do not account for the fact that future availability of fish oil may not be adequate to sustain such practices in the future.

The third approach is the one taken in the present study: increasing the conversion of ALA present in currently available vegetable oils to EPA and DHA in the fish. We have found that completely replacing fish oil with linseed oil can result in similar EPA and DHA levels in fish tissue. As seen in the ELO diet, the addition of the antioxidants vitamin E and BHT and the use of encapsulation may increase the availability of ALA for bioconversion, although the same results may have also been obtained from an encapsulated product containing the same levels of ALA. Whether this is due to the employment of encapsulation or simply the fact that even at lower levels, the ALA present in the ELO was high enough to reach the requirement for fatty acid bioconversion is yet to be determined. Further research is required to determine whether or not antioxidants increase the bioavailability of low levels of ALA and if so, the effects of antioxidant type and dose on the conversion of ALA in flax oil and other oils which are rich in ALA such as canola, mustard and camelina oils. Studies investigation other coating materials for encapsulation that do not affect the fatty acid composition of the diet and their effect on the mortality rate and body fat composition of the resulting fish may also

prove useful.

Clearly, there is no immediate solution for this problem. Rather, a collaborative effort using all three of these approaches must be made to develop a comprehensive approach to reducing the reliance of the aquaculture industry on fish oil for the production of domesticated fish. The incorporation of the research obtained in this study may prove useful in this effort.

6. ACKNOWLEDGEMENTS

This project was funded by the Flax Council of Canada and the Natural Sciences and Engineering Research Council of Canada. Thanks to Dr. R. K. York of the Freshwater Institute in Winnipeg, Manitoba for her guidance in useful methodologies for the preparation of fish samples for sensory analysis.

7. REFERENCES

Alam, M. G. M., Tanaka, A., Allinson, G., Laurenson, L. J. B., Stagnitti, F. and Snow, E. T. 2002. A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of Lake Kasumigaura, Japan. Ecotox Environ Safe. **53:** 348-354.

Alasalvar, C., Taylor, K. D. A., Zubcov, E., Shahidi, F. and Alexis, M. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty aid and trace mineral composition. Food Chem. **79:** 145-150.

Andersen, F., Lygren, B., Maage, A. and Waagbø, R. 1998. Interaction between two dietary levels of iron and two forms of ascorbic acid and the effect on growth, antioxidant status and some non-specific immune parameters in Atlantic salmon (*Salmo salar*) smolts. Aquaculture. **161:** 437-451.

Antunes, P. and Gil, O. 2004. PCB and DDT contamination in cultivated and wild sea bass from Ria de Aveiro, Portugal. Chemosphere. **54:** 1503-1507.

AOAC. 1990. Official Methods of Analysis of AOAC International. 15th edition. Ed. K. Helrick. AOAC Int. Arlington, VA, USA.

AOAC. 1995. Official Methods of Analysis of AOAC International. 16th edition. Ed. P. Cuniff. AOAC Int. Arlington, VA, USA.

AOCS. 1998. Official Methods and Recommended Practices. 5th edition. Ed. D. Firestone. AOCS. Champaign, IL, USA.

Arrigoni, O. and De Tullio, M. C. 2002. Ascorbic acid: much more than just an antioxidant. Biochim Biophys Acta. **1569:** 1-9.

Arzel, J., Lopez, F. X. M., Metailler, R., Stephan, G., Gademer, G. and Guillaume, J. 1994. Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*). Aquaculture **123**:326-37.

Aydin, K. Y., McFarlane, G. A., King, J. R., Megrey, B. A. and Myers, K. W. 2005. Linking oceanic food webs to coastal production and growth rates of Pacific salmon (*Oncorhynchus* spp.), using models on three scales. Deep Sea Res Part II. **52:** 757-780.

Bautista-Teruel, M. N. and Subosa, P. F. 1999. Butylated hydroxytoluene: its effect on the quality of shrimp diet stored at various temperatures and on growth and survival of *Penaeus monodon* juveniles. Aquaculture. **179:** 403-424.

Barham, J. B., Edens, M. B., Fonteh, A. N., Johnson, M. M., Easter, L. and Chilton, F. H. 2000. Addition of eicosapentaenoic acid to gamma-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. J Nutr. **130:** 1925-1931.

Bell, J. G., Henderson, R. J., Tocher, D. R., McGhee, F., Dick, J. R., Porter, A., Smullen, R. P. and Sargent, J. R. 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J Nutr. **132**: 222-230.

Bell, J. G., Henderson, R.J., Tocher, D. R. and Sargent, J. R. 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipids. **39:** 223-232.

Bell, J. G., McEvoy, J., Toucher, D. R., McGhee, F., Campbell, P. J., and Sargent, J. R. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J Nutr. 131: 1535-1543.

Bell, J. G., Tocher, D. R., Henderson, R. J., Dick, J. D. and Crampton, V. O. 2003. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J Nutr. **133:** 2793-2801.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Phys. 37: 911-917.

Boran, G., Karacam, H. and Boran, B. 2006. Changes in the quality of fish oils due to storage temperature and time. Food Chem. **98:** 693-698.

Brenner, R.R., 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res. **20:** 41-47.

Burdge, G. C., Jones, A. E. and Wootton, S. A. 2002. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. Br J Nutr. **88:** 355-363.

Burger, J. and Gochfeld, M. 2005. Heavy metals in commercial fish in New Jersey. Environ Res. **99:** 403-412.

Buzzi, M., Henderson, R. J. and Sargent, J. R. 1997. Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. Comp Biochem Physiol B. **116:** 263-267.

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Izquierdo, M. S. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fat composiion and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. **214:** 253-271.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. and Richardson, R. I. 2006. Flavour perception of oxidation in beef. Meat Sci. **72:** 303-311.

Carubelli, G., Fanelli, R., Mariani, G, Nichetti., S., Crosa. G., Calamari, D. and Fattore, E. 2007. PCB contamination in farmed and wild sea bass (*Dicentrarchus labrax* L.) from a coastal wetland area in central Italy. Chemosphere. **68:** 1630-1635.

Castell, J. D., Sinnhuber, R. O., Wales, J. H. and Lee, D. J. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. J Nutr. **102:** 77-86.

CCAC. 1993. Guide to the care and use of experimental animals. Eds. E.D. Olfert, Cross, B. M. and McWilliams, A. A. Canadian Council on Animal Care. Ottawa, ON, Canada.

CCAC. 2005. Guidelines on the care and use of fish in research, teaching and testing. Canadian Council on Animal Care. Ottawa, ON, Canada.

CFR (Code of Federal Regulations). 2007. Title 21. Food and drugs. USFDA Centre for diseases and radiological health. Rockville, MD, USA.

Chaiyapechara, S., Casten, M. T., Hardy, R. and Dong, R. M. 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Onchorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. Aquaculture. **219:** 715-738.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., O'Keefe, J. H. and Brand-Miller, J. 2005. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr. **81:** 341-54.

Das, U. N. 2006. Essential fatty acids: biochemistry, physiology and pathology. Biotechnol J. **1:** 420-439.

De Francesco, M., Parisi, G., Médale, F., Lupi, P., Kaushik, S and Poli, B. 2004. Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout. Aquaculture. **236:** 413-429.

Dehn, L., Follmann, E. H., Thomas, D. L., Sheffield, G. G., Rosa, C., Duffy, L. K. and O'Hara, T. M. 2006. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci Total Environ. **362:** 103-123.

Deutsch, J. C. 2000. Dehydroascorbic acid. J Chromatogr. **881:** 299-307

Drew, M. D., Ogunkoya, A. E., Janz, D. M. and Van Kessel, A. G. 2007. Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. **267:** 260-268.

Drobna, Z., Zelenka, J., Mrkvicova, E. and Kladroba, D. 2006. Influence of dietary linseed and sunflower oil on sensory characteristics of rainbow trout (*Oncorhynchus mykiss*). Czech J Anim Sci. **51**:475-482.

D'Souza, N., Skonberg, D. I., Camire, M. E., Guthrie, K. E., Malison, J. and Lima, L. 2005. Influence of dietary genistein levels on tissue genistein deposition and on the physical, chemical, and sensory quality of rainbow trout, *Oncorhynchus mykiss*. J Agric Food Chem. **53:** 3631-3636.

Easton, M.D.L., Luszniak, D. and Von der Geest, E. 2002. Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. Chemosphere. **46:** 1053-1074.

Farombi, E. O. and Britton, G. 1999. Antioxidant activity of palm oil carotenes in organic solution: effects of structure and chemical reactivity. Food Chem. **64:** 315-321.

Fernández, J., Pérez-Álvarez, J. A. Fernández-López, J. A. 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chem. **59:** 345-353.

Fonseca-Madrigal, J., Karalazos, V., Campbell, P. J., Bell, J. G. and Tocher, D. R. 2005. Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). Aquacult Nutr. **11:** 241-250.

Fontagné, S., Bazin, D., Brèque, J., Vachot, C., Bernarde, C., Rouault, T. and Bergot, P. 2006. Effects of dietary oxidized lipid and vitamin A on the early development and antioxidant status of Siberian sturgeon (*Acipenser baeri*) larvae. Aquaculture. **257:** 400-411.

Gardiner, N. S. and Duncan, J. R. 1988. Enhanced prostaglandin synthesis as a mechanism for inhibition of melanoma cell growth by ascorbic acid. Prostag Leukotr Ess. **34:** 119-126.

Garg, M. L., Thomson, A. B. R. and Clandinin, M. T. 1990. Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. J Lipid Res. **31:** 271-277.

Geurden, I., Gondouin, E., Rimbach, M., Koppe, W., Kaushik, S. and Boujard, T. 2006. The evaluation of energy intake adjustments and preferences in juvenile rainbow trout fed increasing amounts of lipid. Physiol Behav. **88:** 325-332.

Glushenkova, A. I. and Markman, A. L. 1970. The iodine number and the unsaturation number of fats. Chem Nat Compd. **6:** 671-675.

Goodstine, S. L., Zheng, T., Holford, T. R., Ward, B. A., Carter, D., Owens, P. H. and Mayne, S. T. 2003. Dietary (n-3)/(n-6) fatty acid ratio: possible relationship to premenopausal but not postmenopausal breast cancer risk in U.S. women. J Nutr. **133:** 1409-1414.

Goulas, A. E. and Kontominas, M. G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. Food Chem. **93:** 511-520.

Grant, B. F., Seib. P. A., Liao, M. and Corpron, K. E. 1989. Polyphosphorylated L-ascorbic acid: a stable form of vitamin C for aquaculture feeds. J World Aquacult Soc. **20:** 143-157.

Griel, A. E., Kris-Etherton, P. M., Hilpert, K. F., Zhao, G., West, S. G. and Corwin, R., L. 2007. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. Nutr J. **6:** 2.

Grigorakis, K. 2007. Compositional and organoleptic quality of farmed and wild gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors affecting it: a review. Aquaculture. **272:** 55-75.

Gu, W. and Hecht, N. B. 1996. Developmental expression of glutathione peroxidase, catalase, and manganese superoxide dismutase mRNAs during spermatogenesis in the mouse. J Androl. **17:** 256-262.

Guillén, M. D. and Cabo, N. 2002. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. Food Chem. **77:** 503-510.

Haard, N. F. 1992. Control of chemical composition and food quality attributes of cultured fish. Food Res Int. **25:** 289-307.

Harris, W. S, Poston, W. C. and Haddock, C. K. 2007. Tissue *n*-3 and *n*-6 fatty acids and risk for coronary heart disease events. Atherosclerosis. **193:** 1-10.

Harrison, N. Wearne, S., Gem, M. G. de M., Gleadle, A., Startin, J., Thorpet, S., Wright, C., Kelly, M., Robinson, C., White, S., Hardy, D. and Edinburgh, V. 1998. Time trends in human dietary exposure to PCDDs, PCDFs and PCBs in the UK. Chemosphere. **37:** 1657-1670.

Hevrøy, E. M., Sandnes, K. and Hemre, G. –I. 2004. Growth, feed utilization, appetite and health in Atlantic salmon (Salmo salar L.) fed a new type of high lipid fish meal, Sea Grain®, processes from various pelagic marine fish species. Aquaculture. **235:** 371-392.

Holman, R. T. 1986. Control of polyunsaturated acids in tissue lipids. J Am Coll Nutr. **5:** 183-211.

Horrobin, D. F. 1981. Loss of delta-6-desaturase activity as a key factor in aging. Med Hypotheses. **7:** 1211-1220.

Howe, P., Meyer, B., Record, S. andBaghurst, K. 2006. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition. **22:** 47-53.

Imai, H. and Nakagawa, Y. 2003. Biological significance of phospholipids hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. Free Radical Bio Med. **34:** 145-169.

Inoue, T., Ando, K. and Kikugawa, K. 1998. Specific determination of malonaldehyde by *N*-methyl-2-phenylindole or thiobarbituric acid. JAOCS. **75:** 597-600.

IPCS (International Programme on Chemical Safety). 1992. Polychlorinated biphenyls and terphenyls. Environmental health criteria document 140. WHO. Geneva, Switzerland.

Isbell, T. A., Abbott, T. P. and Carlson, K. D. 1999. Oxidative stability index of vegetable oils in binary mixtures with meadowfoam oil. Ind Crops Prod. **9:** 115-123.

Izquierdo, M. S., Montero, D., Robaina, L., Caballero, M. J., Rosenlund, G. and Ginés, R. 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long time period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture. **250:** 431-444.

Kahl, R. and Hildebrandt, A. G. 1986. Methodology for studying antioxidant activity and mechanisms of action of antioxidants. Food Chem Toxicol. **24:** 1007-1014.

Kanazawa, A., Teshima, S. and Ono, K. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp Biochem Physiol B. **63:** 295-298.

Kanner, J. and Rosenthal, I. 1992. An assessment of lipid oxidation in food. Pure Appl Chem. **64:** 1959-1964.

Kaya, A., Tekin, A. R. and Öner, M. D. 1993. Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage. Lebensm Wiss Technol. **26:** 464-468.

Kelly, K. A., Havrilla, C. M., Brady, T. C., Abramo, K. H. and Levin, E. D. 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ Health Perspect. **106:** 375-384.

Keough, K. M. V. and Kariel, N. 1987. Differential scanning colorimetric studies of aqueous dispersions of phosphotidylcholins containing two polyenoic chains. Biochim Biophys Acta. **902:** 11-18.

Kiecolt-Glaser, J. K., Belury, M. A., Porter, K., Beversdorf, D. Q., Lemeshow, S. and Glasser, R. 2007. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. Pyschosom Med. **69:** 217-224.

Kim, M., Kim, S., Yun, S., Lee, M., Cho, M., Park, J., Son, S. and Kim, O. 2004. Comparison of seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat. Chemosphere. **54:** 1533-1538.

Kishino, T., Watanabe, K., Urata, T., Takano, M., Uemura, T., Nishikawa, K., Mine, Y., Matsumoto, M., Ohtsuka, K., Ohnishi, H., Mori, H., Takahashi, S., Ishida, H. and Watanabe, T. 2008. Visceral fat thickness in overweight men correlates with alterations in serum fatty acid composition. Clin Chim Acta. **398:** 57-62.

Kragballe, K., Voorhees, J. J. and Goetzl, E. J. 1987. Inhibition by leukotriene B_5 of leukotriene B_4-induced activation of human keratinocytes and neutrophils. J Invest Dermatol. **88:** 555-558.

Kris-Etherton, P. M., Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R. L., Zhao, G. and Etherton, T. D. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr. **71:** 179S-188S.

Labrinea, E. P., Thomaidis, N. S. and Georgiou, C. A. 2001. Direct olive oil anisidine value determination by flow injection. Anal Chim Acta. **448:** 201-206.

Laing, B. M., Schlueter, D. L. and Labuza, T. P. 1978. Degradation kinetics of ascorbic acid at high temperature and water activity. J Food Sci. **43:** 1440-1443.

Lauzurica, S., de al Fuente, J., Diaz, M. T., Álvarez, I., Pérez, C. and Cañeque, V. 2005. Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. Meat Sci. **70:** 639-646.

Lee, S. H., Stephens, J. L. and Englund, P. T. 2007. A fatty-acid synthesis mechanism specialized for parasitism. Nat Rev Microbiol. **5:** 287-297.

Leger, C, Gatesoupe, F., Metailler, R., Luquet, P. and Fremont, L. 1979. Effect of dietary fatty acids differing by chain lengths and omega series on the growth and lipid composition of turbot *Scophthalmus maximus* L. Comp Biochem Physiol B. **64:** 345-350.

Levin, G., Duffin, K. L., Obukowicz, M. G., Hummert, S. L., Fujiwara, H., Needleman, P. and Raz, P. 2002. Differential metabolism of dihomo-gamma-linolenic acid and arachidonic acid by cyclo-oxygenase-1 and cyclo-oxygenase-2: implications for cellular synthesis of prostaglandin E_1 and prostaglandin E_2. Biochem J. **365:** 489-496.

Li, P., Wang, X., Hardy, R. W. and Gatlin, D. M. 2004. Nutritional value of fisheries by-catch and by-product meals in the diet of red drum (*Scaenops ocellatus*). Aquaculture. **236:** 485-496.

Li, S., Yan, T., Yang, J., Oberley T. D. and Oberley, L. W. 2000. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res. **60:** 3927-3939.

Liu, K. K. M., Barrows, F. T., Hardy, R. W. and Dong, F. M. 2004. Body composition, growth performance, and product quality of rainbow trout (Oncorhynchus mykiss) fed diets containing poultry fat, soybean/corn lecithin, or menhaden oil. Aquaculture. **238:** 309-328.

Lukaszewicz, M., Szopa, J. and Krasowska, A. 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. Food Chem. **88:** 225-231.

Maule, A. G., Gannam, A. L. and Davis, J. W. 2007. Chemical contaminants in fish feeds used in federal salmonid hatcheries in the USA. Chemosphere. **67:** 1308-1315.

McLaren, B. A., Keller, E., O'Donnell, D. J. and Elvehjem, C. A. 1947. The nutrition of rainbow trout. 1. Studies of vitamin requirements. Arch Biochem Biophys. **15:** 169-178.

McLennan, P. L. and Dallimore, J. A. 1995. Dietary canola oil modifies fatty acids and inhibits cardiac arrhythmias in rats. J Nutr. **125:** 1003-1009.

Meyer, A., Kirsch, H., Domergue, F., Abbadi, A., Sperling, P., Bauer, J., Cirpus, P., Zank, T. K., Moreau, H., Roscoe, T. J., Zähringer and Heinz, E. 2004. Novel fatty acid elongases and their use for the reconstitution of docosahexaenoic acid biosynthesis. J Lipid Res. **45:** 1899-1909.

Mozaffarian, D., Ascherio, A., Hu, F. B., Stampfer, M. J., Willett, W. C., Siscovick, D. S. and Rimm, E. B. 2005. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation. **111:** 166-173.

Nakamura, M. T. and Nara, T.Y. 2003. Essential fatty acid synthesis and its regulation in mammals. Prostag Leukotr Ess. **68:** 145-150.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press. Washington, DC, USA.

New, M. B. and Wijkstrom, U. N. 2002. Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap. FAO Fisheries Circular (975). FAO, Rome, Italy.

Olsson, G. B., Olsen, R. L., Carlehög, M. and Ofstad, R. 2003. Seasonal variations in chemical and sensory characteristics of farmed and wild Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture. **217:** 191-205.

Owen, J. M., Adron, J. W., Middleton, C. and Cowey, C. B. 1975. Elongation and desaturation of dietary fatty acids in turbot *Scophthalmus maximus* L., and rainbow trout, *Salmo gairdnerii* Rich. Lipids. **10:** 528-531.

Packer, J. E., Slater, T. F. and Willson, R. L. 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature. **278:** 737-738.

Raisz, L. G., Alander, C. B. and Simmons, H. A. 1989. Effects of prostaglandin E3 and eicosapentaenoic acid on rat bone in organic culture. Prostaglandins. **37:** 615-625.

Regost, C., Arzel, J., Cardinal, M., Rosenlund, G. and Kaushik, S. J. 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in Turbot (*Psetta maxima*) 2. Flesh quality properties. Aquaculture. **220:** 737-747.

Rhee, K. S. and Myers, C. E. 2003. Sensory properties and lipid oxidation in aerobically refrigerated cooked ground goat meat. Meat Sci. **66:** 189-194.

Riebroy, S. Benjakul, S., Visessanguan, W. and Tanaka, M. 2007. Effect of iced storage of bigeye snapper (*Priacanthus tayenus*) on the chemical composition, properties and acceptability of som-fug, a fermented Thai fish mince. Food Chem. **102:** 270-280.

Rinchard, J., Czesny, S. and Dabrowski, K. 2007. Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. Aquaculture. **264:** 363-371.

Rinchard, N., Kaushik, S., Larroquet, L., Panserat, S. and Corraze, G. 2006. Replacing dietary fish oil by vegetable oil has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). Br J Nutr. **96:** 299-309.

Rørå, A. M. B., Kvåle, A., Mørkøre, T., Rørvik, K., Steien, S. H. and Thomassen, M. S. 1998. Process yield, colour and sensory quality of smoked Atlantic salmon (Salmo salar) in relation to raw material characteristics. Food Res Int. **31:** 601-609.

SAS (SAS Institute Inc). 2004. SAS/ACCESS® 9.1 Interface to PeopleSoft: user's guide. SAS Institute Inc. Cary, NC, USA.

Sau, S. K., Paul, B. N., Mohanta, K. N. and Mohanty, S. N. 2004. Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. Aquaculture. **240:** 359-368.

Schmidt, E. B., Arnesen, H., de Caterina, R., Rasmussen, L. H. and Kristensen, S. D. 2005. Marine n-3 polyunsaturated fatty acids and coronary heart disease. Part I. Background, epidemiology, animal data, effects on risk factors and safety. Thromb Resnb. **115:** 163-170.

Schecter, A., Dellarco, M., Päpke, O. and Olson, J. 1998. A comparison of dioxins, dibenzofurans and coplanar PCBs in uncooked and broiled ground beef, catfish and bacon. Chemosphere. **37:** 1723-1730.

Shahidi, F. and Wanasundara, P. K. J. P. D. 1992. Phenolic antioxidants. Crit Rev Food Sci. **32:** 67-103.

Shepherd, C.J., Pike, I.H. and Barlow, S.M. 2005. Sustainable feed resources of marine origin. EAS Special Publication No. 35. European Aquaculture Society. Oostende, Belgium. 59-66.

Sidani, M. and Ziegler, C. 2008. Preventing heart disease: who needs to be concerned and what to do. Prim Care. **35:** 589-607.

Simopoulos, A. P. 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr. **21:** 495-505.

SPSS (SPSS Inc). (2005). SPSS base 14.0 user's guide. SPSS Inc. Chicago, IL, USA.

Startin, J. R., Rose, M. and Offen, C. 1989. Analysis of PCDDs and PCDFs in human milk from the UK. Chemosphere. **19:** 985-988.

Tan, C. P., Che Man, Y. B., Selamat, J. and Yusoff, M. S. A. 2002. Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. Food Chem. **76:** 385-389.

Tavella, M., Peterson, G., Espeche, M., Cavallero, E., Cipolla, L., Perego, L. and Caballero, B. 2000. Trans fatty acid content of a selection of foods in Argentina. Food Chem. **69:** 209-213.

Taylor, B. J. and Walsh, M. K. 2002. Development and sensory analysis of a textured whey protein meatless patty. J. Food Sci. **67:** 1555-1558.

Tawfik, M. S. and Huyghebaert. 1999. Interaction of packaging materials and vegetable oils: oil stability. Food Chem. 64: 451-459.

Tocher, D. R., Bell, J. G., MacGlaughlin, P., McGhee, F. and Dick, J. R. 2001. Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. Comp Biochem Physiol B. **130:** 257-270.

Tocher, D. R., Fonseca-Madrigal, J., Dick, J. R., Ng, W., Bell, J. G. and Campbell, P. J. 2004. Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol B. **137:** 49-63.

Tsai, P., Ko, Y., Huang, W., Liu, H. and Guo, Y. 2007. Increased liver and lupus mortalities in 24-year follow-up of the Taiwanese people highly exposed to polychloringated biphenyls and dibenzofurans. Sci Total Environ. **374:** 216-222.

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N. and Dumoulin, E. 2005. Oil encapsulation by spray drying and fluidised bed agglomeration. Innov Food Sci Emerg **6:** 29-35.

Türkmen, M., Türkmen, A., Tepe, Y., Ates, A. and Gökkus, K. 2008. Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean seas: twelve fish species. Food Chem. **108:** 794-800.

Ulu, H. 2004. Evaluation of three 2-thiobarbituric acid methods for the measurement of lipid oxidation in various meats and meat products. Meat Sci. **67:** 683-687.

Ureña, R., Peri, S., del Ramo, J. and Torreblanca, A. 2007. Metal and metallothionein content in tissues from wild and farmed *Anguilla anguilla* at commercial size. Environ Int. **33:** 532-539.

Ursin, V. M. 2003. Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. J Nutr. **133:** 4271-4274.

Vetrano, A. M., Heck, D. E., Mariano, T. M., Mishin, V., Laskin, D. L. and Laskin, J. D. 2005. Characterization of the oxidase activity in mammalian catalase. J Biol Chem. **280:** 35372-35381.

Visentainer, J. V., de Souza, N. E., Makoto, M., Hayashi and Franco, M. R. B. 2005. Influence of diets enriched with flaxseed oil on the alpha–linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*). Food Chem. **90:** 557-560.

Waagbø, R., Sandnes, K., Torrissen, O. J. Sandvin, Andnes, S. and Lie, Ø. 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chem. **46:** 361-366.

Wanasundara, U. N. and Shahidi, F. 1994. Stabilization of canola oil with flavenoids. Food Chem. 50: 393-396.

Williams, G. M., Iatropoulos, M. J. and Whysner, J. 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chem Toxicol. **37:** 1027-1038.

Williams, T. J. and Peck, M. J. 1977. Role of prostaglandin-mediated vasodilation in inflammation. Nature. **270:** 530-532.

Wagner, K. H. and Elmadfa, I. 2000. Effects of tocopherols and their mixtures on the oxidative stability of olive oil and linseed oil under heating. Eur J Lipid Sci Tech. **102:** 624-629.

Yang, P., Chan, D., Felix, E., Cartwright, C., Menter, D. G., Madden, T., Klein, R. D., Fischer, S. M. and Newman, R. A. 2004. Formation and antiproliferative effect of prostaglandin E_3 from eicosapentaenoic acid in human lung cancer cells. J Lipid Res. **45:** 1030-1039.

Yoneda, T., Saitou, K., Mizushige, T., Matsumura, S., Manabe, Y., Tsuzuki, S., Inoue, K. and Fushiki, T. 2007. The palatability of corn oil and linoleic acid to mice as measured by short-term two-bottle choice and licking tests. Physiol Behav. **91:** 304-309.

Young, I. S. and McEneny, J. 2001. Lipoprotein oxidation and atherosclerosis. Biochem Soc Trans. **29:** 358-362.

Zanini, S. F., Torres, C. A., Bragagnolo, N., Turatti, J. M., Silva, M. G., Zanini, M. S. 2004. Effect of oil sources and vitamin E levels in the diet on the composition of fatty acids in rooster thigh and chest meat. J Sci Food Agric. **84**:672-682.

Zheng, X., Seiliez, I., Hastings, N., Tocher, D. R., Panserat, S., Dickson, C. A., Bergot, P. and Teale, A. J. 2004. Characterization and comparison of fatty acyl Δ6 desaturase cDNAs from freshwater and marine teleost fish species. Comp Biochem Physiol B. **139:** 269-279.

Appendix 1 Trained and untrained panelist information sheet

Date_____

Participant Number_____

Gender

☐ Male ☐ Female

Age

☐ 18-25 ☐ 26-34 ☐ 35-44 ☐ 45-54 ☐ 55-65

Do you smoke?

☐ Yes ☐ No

How often do you eat fish?

☐ Never ☐ <Once a Month ☐ Once a Month ☐ >Once a Month

☐ Once a Week ☐ >Once a Week ☐ Every Day

What type(s) of fish do you normally eat?

☐	☐	☐	☐	☐
Trout	Salmon	Tuna	Haddock, Whitefish	Snapper
☐	☐	☐	☐	☐
Catfish	Carp	Tilapia	Shellfish	Other

Other (please list, use back of page if necessary): _____

Appendix 2 Trained panelist training procedure

On the first day of training, only two samples were presented to each panelist. One sample was a piece of trout that was raised at Wild West Steelhead; the second was a piece of trout that was raised at the PARC. The fish that were raised at the PARC were originally purchased from the hatchery at Wild West Steelhead so both samples originated from the same genetic stock. The fish samples presented to the panelists were quite large (approx. 3 cm^3) on the first day of training. As the training went on, the sample size became progressively smaller (to approx. 1.5 cm^3).

On the second day of training, three samples were presented to each panelist. One sample was a piece of trout that was raised at Wild West Steelhead. One sample was a piece of tilapia (Captain High Liner ®). The third sample was a piece of monkfish.

The third day of training involved four samples being presented to each panelist. Two pieced of trout that were raised at Wild West Steelhead were presented to each panelist but they were in fact true duplicates (the same piece of fish cut in half). Two spiked pieces of fish were also given to the panelists. They were both pieces of trout that were raised at Wild West Steelhead. One was spiked by adding the contents of a capsule of fish oil to the sample. The other was spiked by adding the contents of a capsule of flax oil to the sample.

Four samples were presented to each panelist on the fourth day of training. The samples were: a piece of trout that was raised at Wild West Steelhead, a piece of catfish, a piece of tilapia (Captain High Liner ®), and a piece of tilapia that was spiked with corn. The method used to spike the tilapia with corn is as follows: rather than steaming the fish

in the aluminum foil packet like all of the other samples, the piece of fish was cooked in a pot consisting of corn niblets and water at a ratio of 1:3 and blended for one minute. The sample was cooked for the same amount of time as the other samples. Once cooked, the fish was wrapped in aluminum foil to look as if this was how it was cooked and presented to the panelists along with the other fish samples.

On the fifth, sixth and seventh days of training, the same samples were presented to each panelist (although in varying orders) each day. The results obtained from these three days were used to determine which 12 panelists were to remain on as panelists during the testing portion of this trial. Six samples were presented to each panelist per day. The samples used were two pieces of trout that were raised at Wild West Steelhead (true duplicate) one piece of trout that was raised at the PARC, one piece of catfish, one piece of trout that was spiked with fish oil and one piece of fish that was spiked with flax oil (both raised at Wild West Steelhead).

Appendix 3 Sensory evaluation of cooked rainbow trout fillets by trained panel

Date_____ Participant Number_____

Sample Code_____

Please take a bite of cracker and a drink of water before beginning. Feel free to list any comments on the back of this page.

	8	7	6	5	4	3	2	1
TEXTURE: Initial tenderness	Extremely tender	Very tender	Moderately tender	Slightly tender	Slightly tough	Moderately tough	Very tough	Extremely tough
Overall Tenderness	Extremely tender	Very tender	Moderately tender	Slightly tender	Slightly tough	Moderately tough	Very tough	Extremely tough
Juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
FLAVOUR: Flavour intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly mild	Moderately mild	Very mild	Extremely mild
Flavour desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Presence of off-flavours	Extremely high	Very high	Moderately high	Slightly high	Slightly low	Moderately low	Very low	Extremely low
Circle any off-flavours you detect	Fishy	Painty	Oily	Corn	Chickeny	Earthy/musty	Rancid	Nutty
Under each of the off-flavours you detected, rank them between 8 (high) and 1 (low)								
OVERALL PALATABILITY	Extremely palatable	Very palatable	Moderately palatable	Slightly palatable	Slightly unpalatable	Moderately unpalatable	Very unpalatable	Extremely unpalatable

Appendix 4 Sensory evaluation of cooked rainbow trout fillets by untrained panel

Sample Code_____ Date_____

Participant Number_____

- **Please take a bite of cracker and a drink of water before each sample. Lemon water is provided to cleanse the palate if desired.**
- **Please evaluate the sample based on its individual characteristics.**

Aroma

☐	☐	☐	☐	☐	☐
Very Mild	Moderately Mild	Slightly Mild	Slightly Strong	Moderately Strong	Very Strong

Acceptability of Aroma

☐	☐	☐	☐	☐	☐
Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much

Flavour

☐	☐	☐	☐	☐	☐
Very Bland	Moderately Bland	Slightly Bland	Slightly Intense	Moderately Intense	Very Intense

Acceptability of Flavour

☐	☐	☐	☐	☐	☐
Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much

Texture

☐	☐	☐	☐	☐	☐
Very Firm	Moderately Firm	Slightly Firm	Slightly Soft	Moderately Soft	Very Soft

Acceptability of Texture

☐	☐	☐	☐	☐	☐
Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much

Juiciness

☐	☐	☐	☐	☐	☐
Very Dry	Moderately Dry	Slightly Dry	Slightly Juicy	Moderately Juicy	Very Juicy

Acceptability of Juiciness

☐	☐	☐	☐	☐	☐
Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much

Overall Acceptability

☐	☐	☐	☐	☐	☐
Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much

Would you buy this product?

☐	☐	☐	☐
Definitely No	Probably No	Probably Yes	Definitely Yes

Comments (use back of page if necessary)